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ISFEV 2018

7-10 OCTOBER

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Prevalence of human enteric viruses in shellfish from Galicia (nw Spain): a 20 years perspective

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Objective

Galician (NW Spain) coasts, with their characteristics fiord-like rías, constitute very favourable environments for shellfish growth and culture. In fact, Galicia is one of the most important mussel producers in the world. The proximity of the harvesting areas to cities and villages and the anthropogenic activities in these estuaries make sometimes possible their contamination with material of faecal origin. Routine official controls, based on *Escherichia coli* as established in current regulation, of the Galician harvesting areas have implemented for a long time which have been revealed as useful indicator for bacterial contaminants. However, this is not the case for enteric viruses. The aim of this review is to offer a picture on the situation of different harvesting areas in Galicia from a virological standpoint.

Methods

A recompilation of results obtained in the last 20 years is presented, including data for the well known agents Norovirus (NoV) and hepatitis A virus (HAV), together with data obtained for emerging viral hazards, including Sapovirus (SaV), hepatitis E virus (HEV) and Aichivirus (AiV).

Results

Differences in viral prevalence related to diverse characteristics of the harvesting areas, viral genotype distribution and shifts, as well as epidemiological links between environmental and clinical strains will be presented and discussed.

Conclusions

The compilation of these historical data could be matter of consideration for future decisions by the competent authorities for a better management of Galician shellfish growing areas.

The European union reference laboratory for foodborne viruses

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Introduction

The increasing awareness of the transmission of foodborne viruses through foods and water has led the EU commission, DG SANTE health and food safety of the European Union to decide to constitute a new European Union Reference Laboratory (EURL) for foodborne viruses.

Objectives

The main objective for such a reference laboratory is to harmonize methods for the detection and quantification of foodborne viruses in foods and water.

Results

From January 2018 the National Food Agency in Sweden is the designated laboratory to carry out this task. The main tasks for the EURL and forthcoming activities will be described:

- To ensure availability and use of high quality methods and to ensure high quality performance by National Reference Laboratories (NRLs)
- To provide scientific and technical assistance to NRLs
- To provide scientific and technical assistance to the European Commission and other organizations
- To provide and keep a list of reagents and reference collections

A work program has been carried out to implement the main tasks for the EURL.

Conclusions

We hope that the establishment of an NRL network for foodborne viruses in Europe will lead to improved laboratory capacity and increased awareness of foodborne virus transmission in general.

Occurrence and diversity of human enteroviruses circulating in Luxembourg

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Purpose and Objective.

Human enteroviruses (HEVs) are RNA viruses that are responsible for both mild gastroenteritis and respiratory illnesses as well as diseases such as meningitis and myocarditis. Since infected individuals shed enterovirus in faeces, surveillance of municipal wastewater can reveal the diversity of HEVs circulating in human population. HEVs can occur in high concentrations in wastewater and contaminate receiving environmental waters, constituting a major public health concern worldwide. In this study, we investigated and compared the occurrence and the diversity of infectious HEVs in Luxembourg from September 2017 to December 2017, both in wastewater treatment plants (WWTPs) and in faeces samples of people presented clinical symptoms.

Method.

Influent samples from the seven main WWTPs of Luxembourg were collected monthly. An integrative sampling strategy was applied to cover a 24-h period. Wastewater samples (n=28) were concentrated using an optimised Celite protocol before inoculation of BGM cells following by a RT-qPCR detection. Faecal samples (n=87) were submitted to RNA extraction following by a RT-qPCR detection. A high throughput sequencing assay (Illumina technology) are under development to solve the diversity of HEVs occurring in the gathered samples.

Results and Conclusions

Seventeen of the twenty-eight wastewater samples collected (61%) were CPE positive on the BGM cells, and sixteen were confirmed to be HEVs by RT-qPCR. Among the clinical samples, thirteen of the eighty-seven faeces samples (15%) were declared positive for HEVs by the RT-qPCR.

Data on viral detection confirmed the circulation of HEVs in Luxembourg. The typing information obtained by the next-generation sequencing will allow accurate knowledge of the HEV species diversity and also to perform a comparative study between the clinical and the wastewater samples.

Human sapovirus in wastewaters from Tunisia

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Objectives

Sapovirus is a genus of enteric viruses that cause acute gastroenteritis worldwide and is shed at high concentrations with feces into wastewater. This study analyzed the incidence of human sapovirus in four wastewater treatment plants from Tunisia during a of 13 months period, and also provides an estimation of the sapovirus density in wastewater using Bayesian algorithms with left-censored data sets.

Methods

Total of 218 samples (2 L of wastewater) from influent and effluent points of the plants were obtained every two weeks. Viral concentration was carried out by the adsorption-elution method. Detection and quantification were carried out using RT-qPCR, and positive samples were amplified by RT-nested PCR for genotyping. Bayesian estimation algorithm was applied to estimate posterior predictive distributions of sapovirus density in influent and effluent using left-censored data sets. Log ratio posterior distribution was also calculated by dividing two posterior predictive distributions of virus density in influent and effluent samples.

Results

Sapovirus prevalence was 39.9%, with 61 samples detected in untreated water and 26 in processed water. Mean quantification for the study was 7.0 x 10^5 genomic copies/L wastewater. Genotype GI.2 was the most observed. The mean value of log sapovirus concentration in untreated wastewater ranged between 2.7 and 4.5 logs. A virus removal efficiency of 0.2 log was calculated for the one plant, as the log ratio posterior distributions between untreated and treated wastewater.

Conclusions

The present study presents novel findings on the prevalence, seasonality and genotype distribution of sapovirus in Tunisia. A Bayesian estimation of the posterior predictive distribution (“left-censored” data) was employed to solve methodological problems related with the limit of quantification of the qPCR. This approach can be useful for the future development of microbial risk assessment procedures for wastewater.

Evaluation and implementation of mitigation strategies for viral pathogens in the berry supply chain

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Introduction

Foodborne viruses have been associated with many foodborne illness outbreaks linked to fresh and frozen berries worldwide. In 2014, our supply chain of frozen berries was associated with cases of Hepatitis A.

Objectives

We launched a company-wide project to ensure that such incidents would not recur which included (i) monitoring of foodborne viruses in berries, (ii) evaluation of alternative technologies for the inactivation of viral pathogens on berries and (iii) translation of scientific data into realistic and easily implementable guidance for auditors, suppliers and growers.

Methods

(i) Over the time period from 2009 to 2016, 2'015 samples were analyzed for HAV, NoV GI and GII in various berries sourced from different countries using a real-time PCR method based on ISO/TS 15216:2013. (ii) The efficacy of alternative technologies in removal/inactivation of viruses was evaluated using TCID50 or plaque assays on HAV HM-175, MNV S99 or MNV-1 and MS2 bacteriophage. (iii) The best practices were developed in close collaboration with farmers and suppliers to convert theoretical requirements into easily understood and implementable recommendations.

Results

(i) Seven positive signals were identified from 2'015 samples analyzed. (ii) The gaseous ozone study showed that a 30 min treatment with 6 % wt/wt gaseous ozone delivered reductions on fresh strawberries of 1.8 and 3.3 log10 for MNV-1 and MS2, respectively. (iii) Two training booklets entitled "'Minimizing the risk of microbial contamination in primary production of berries" and "Washing of produce: Guidance to minimize the microbiological risk" were developed and disseminated through external partners including GFSI, PROFEL and local authorities.

Conclusions

Feedback received from suppliers and famers show the successful spreading and implementation of our food safety recommendations directly in the field, where food safety starts. Ozone gas has the potential to address the issue of berries which cannot be washed.

Inactivation of HAV, MNV and MS2 on onions, raisins and blueberries during mild drying processes

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Objectives

The purpose of this study was to understand the different levels of inactivation of foodborne viruses which may be achieved in different drying processes applied routinely in the food industry (drying of onions, heat treatment of dried raisins and candying of blueberries).

Methods

Fresh sliced onions, dried raisins and defrosted blueberries were spot-inoculated with Hepatitis A virus (HAV), Murine Norovirus (MNV) and MS2 bacteriophage and treated up to 45 min at 60°C, up to 60 min at 62 °C and sugar infused 15 hours at temperatures ranging from 23 to 55°C followed by a drying step for 60 min at 100°C (candying), respectively. After treatment, HAV and MNV were recovered following the ISO-15216 method and quantified using $TCID_{50}$. MS2 bacteriophages were quantified using the ISO-10705-1 method.

Results

Sliced onions treated at 60°C for 45 min achieved > 4 log_{10} reduction for MNV and < 2 log_{10} for MS2 and HAV. Dried raisins treated at 62°C for 60 min reduced MNV and MS2 by > 4 log_{10}, and HAV by < 1 log_{10}. During the candying of blueberries, sugaring at 45°C showed reductions of > 3 log_{10}, > 4 log_{10}, and < 2 log_{10} for MNV, MS2 and HAV, respectively. The air-drying of the sugared blueberries at 100°C for 1 hour delivered around 3, 6 and 2 log_{10} reductions of MNV, MS2 and HAV, respectively.

Conclusions

These data show that mild processing can bring acceptable levels of inactivation towards viruses when specific processing parameters are applied. However, this study also shows that the industry is lacking appropriate virus surrogates for validation studies at industrial scale.

Green tea extract to control enteric viruses in food

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Introduction

Green tea extract (GTE), obtained from cultivated evergreen tea plant (*Camellia sinensis* L), has been widely used in various food and beverage applications due to its diverse health benefits, including antimicrobial properties, derived from its polyphenolic rich composition. Chemically, GTE mainly contains catechins which have shown antiviral activity against human norovirus (NoV) surrogates and hepatitis A virus (HAV).

Objectives

The purpose of this work was to investigate the antiviral activity of GTE over time at different concentrations, temperatures and pHs. Additionally, GTE was evaluated as a natural disinfectant for food-contact surfaces and incorporated within edible films and coatings.

Methods

GTE powder (Naturex SA, France) was dissolved in PBS (pH 5.5, 7.2 and 8.5) and evaluated at different concentrations (0.5 and 5 mg/ml), temperatures (10, 25 and 37 °C) and aging conditions on HAV and murine norovirus infectivity. Additionally, the effect of aged-GTE was also evaluated on NoV GI suspensions by an *in-situ* capture-RT-qPCR (ISC-RT-qPCR) method based on porcine gastric mucine (PGM).

GTE was further evaluated as food-contact surface sanitizer based on ISO 13697:2001 standard (ISO/TS 13697:2001) and incorporated on alginate films and coatings developed through the solvent casting method. Finally, efficacy of GTE-coatings were investigated in strawberries and raspberries artificially contaminated with MNV and HAV.

Results

The obtained results revealed that GTE was very effective in inactivating MNV and HAV at neutral and alkaline pH but was ineffective at pH 5.5, fact which was partially correlated to the increase in some polyphenolic compounds (specifically in theasinensin A and B in alkaline conditions). Additionally, storage of the solutions for 24 h at various pH conditions significantly increased their antiviral activity. Our study indicated that NoVs are very sensitive to aged-GTE treatment at 37 ºC since GTE at 5 mg/ml prevented NoV GI binding to PGM, while at 0.5 mg/ml some viral particles were still able to bind its receptors.

A GTE solution of 5 mg/ml prepared under the optimal conditions (24 h storage and pH 7.2) was applied only 15 min on stainless steel and glass surfaces for sanitizing purposes, showing a reduction of more than 1.5 log $TCID_{50}$/ml of MNV and HAV infectivity.

Finally, challenge tests on strawberries and raspberries showed that GTE-coatings highly reduced the infectivity of MNV and HAV depending on the storage conditions.

Assessment of novel approaches to investigate norovirus persistency in the aquatic environment

Kata Farkas, Tim J. Fitzmaurice, Ian G. Goodfellow, James E McDonald, Shelagh K Malham, Davey L. Jones

Bangor University, Wales England

Objectives

The aim of this study was to explore the usefulness of different methods, including a stem-cell-derived enteroids assay (SDEA), for describing viral infectivity and persistency in aquatic environments. The primary focus of the study was human norovirus, although, the usefulness of murine norovirus as a surrogate and human adenovirus as a proxy indicator were also investigated.

Methods

In this study, we spiked river water samples with known concentrations of human and murine noroviruses and with human adenovirus. The samples were aliquoted into dialysis tubes, placed in the river Llugwy at Betws-y-Coed, North Wales, UK and sampled regularly for two weeks. The samples were analyzed using direct real-time (RT-)PCR and three approaches to assess viral integrity: RNase treatment and porcine gastric mucin (PGM) assay for noroviruses and DNase treatment for adenovirus. Selected samples were also analyzed using the SDEA to assess norovirus infectivity and tissue culturing with HEK 293 cells to address adenovirus infectivity.

Results

Less than one $\log_{10}$ reduction in viral titers was observed over the period of two weeks using capsid integrity assays and direct detection. The results showed that the capsid integrity assays were all able to eliminate free viral nucleic acids. Our preliminary findings suggest that the infectivity assays can be used on environmental water samples with high viral titers.

Conclusions

Due to the difficulties with the SDEA setup, the use of the assay in routine tests on environmental water samples may not be feasible. However, the current results suggest that the infectivity assays may be suitable for the validation of capsid integrity assays. Once the correlation between norovirus capsid integrity and infectivity is established, the PGM and other approaches may be used to estimate the public health risks associated with water.

Verification of bag-mediated filtration system V2

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Purpose/Objectives

The bag-mediated filtration system (BMFS) is used for environmental surveillance of poliovirus (PV). The original version (v1) was field validated, demonstrating improved PV detection when compared with the World Health Organization's (WHO) standard two-phase separation method. The BMFS v1 was modified (v2), including a new collection bag, filter housing, elution device, and elution and secondary concentration methods. This study compared BMFS v2 (hereafter, v2) with BMFS v1 (hereafter, v1) and the two-phase method for PV detection in Nairobi field samples.

Materials/Methods

Preliminary investigations compared v1 and v2 components and protocols, including the filter housing (n=2; E1), elution (n=3; E2), secondary concentration (n=3; E3), and a combination of these modifications (n=3; E4). An ongoing verification study (March—July 2018) compares matched v1, v2, and two-phase samples from two sites, collected twice monthly (n=5). Samples were assayed for PV by the WHO algorithm.

Results

Preliminary investigations showed Sabin-like PV type 1 (SL1) and type 3 (SL3) detection in the majority of samples. During one sampling event each for E2 and E4, SL1 was detected in the v2 though not the matching v1 sample. Of the six flasks inoculated per sample, v2 resulted in more positive flasks than matching v1 samples; this was statistically significant for E4 (p=0.019 and 0.019 for SL1 and SL3). In the verification study, SL1 and SL3 have been detected in v1 (40% and 80%) and v2 (60% and 80%) samples. The v2 samples have resulted in more positive flasks than matching v1 samples, although this is not statistically significant (p=0.18 and 0.11 for SL1 and SL3). Forthcoming results will be incorporated into ongoing analyses.

Conclusions

Based on available results, v2 has shown similar or improved PV recovery compared to v1. A comprehensive analysis will be completed once all data are available.

KEYNOTE SPEAKER

The use of natural viruses in surface water for the verification of integrity of RO membranes

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Purpose

High pressure membranes are increasingly to produce water for irrigation or drinking. The lack of a fast and easy to implement membrane integrity test method hampers the implementation of these membranes.

Objectives

To identify natural viruses in source water, select suitable candidates for membrane integrity testing, develop qPCR methods and test the integrity of reverse osmosis (RO) membranes in a pilot installation.

Methods

Viruses in water were concentrated, treated with DNase and DNA and RNA were isolated using the PureLink DNA/RNA kit. Next generation sequencing (Illumina HiSeq) was done for DNA and RNA separately. Assemblies were compared with virus sequences in the NCBI nt database. Several virus sequences that were present in the source water of a pilot RO installation, and absent or significantly reduced in the permeate of the RO installation were selected as candidates for integrity testing. qPCR primers were constructed and the Log Removal Value (LRV) of these natural viruses was compared with the LRV of (spiked and cultured) MS2.

Results

Of the assembled scaffolds from source water, only 1.3% of the DNA sequences and 4.5% of the RNA sequences showed a hit with the sequences in the NCBI database. The rest of the sequences is probably ‘viral dark matter’. Since 10x more DNA than RNA was isolated, qPCR primers were developed against selected DNA virus sequences. Application of qPCR in the pilot RO installation that was fed with source water showed that the concentration of the selected natural viruses in the source water was high enough to demonstrate a LRV of at least 7, comparable to the results of the spiked MS2 bacteriophage. Furthermore, after inflicting damage to the RO membrane, both MS2 and the natural viruses detect the damage to the membrane with identical loss of LRV.

Conclusions

So, our method to quantify natural viruses that are present in high concentrations in source water enables testing of RO membrane integrity with high sensitivity (LRV of 7 or more), without the need to add virus (surrogates) to the water and without laborious sample concentration procedures. Implementation of this simple method makes it possible to routinely monitor the integrity of RO membranes in full scale operation with a much higher sensitivity than current methods.

Comparison between RT droplet digital PCR and RT real-time PCR for quantification of noroviruses in oysters

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Introduction

Human noroviruses cause acute gastrointestinal illness and are typically transmitted from person to person or through ingestion of contaminated food or water. Oysters are frequently involved in norovirus outbreaks, but the presence of norovirus RNA in oysters does not necessarily imply a health risk to humans. There is a close link between human illness and high levels of norovirus RNA in oysters. However, low levels of norovirus RNA are more unlikely to be associated with outbreaks. Reliable and reproducible quantification methods are therefore important for outbreak analyses and risk assessment studies.

Objectives

This study optimized and validated RT-ddPCR assays for detection of norovirus genogroups I and II in artificially contaminated oysters and compared them with the standard method RT-qPCR.

Method

Oysters were artificially-contaminated with norovirus GI and GII, extracted, and analysed by RT-ddPCR and RT-qPCR. The two methods were validated by assessing 95 % limits of detection, precision in quantification (repeatability), and quantitative agreement.

Results and Conclusions

RT-ddPCR and RT-qPCR had similar sensitivities in terms of 95 % limits of detection and numbers of detected samples, but RT-ddPCR had generally greater precision (repeatability) in quantification, suggesting that RT-ddPCR can be a promising method for quantification of noroviruses in oysters. However, RT-ddPCR was more expensive and time-consuming than RT-qPCR. Also, RT-ddPCR involves more sample transfer steps, which potentially increases pipetting errors and the risk of contamination. Inter-laboratory comparisons are therefore needed to investigate method reproducibility and other aspects related to the potential of using the method for quantification of noroviruses in oysters.

Infectivity tests coupled with metagenomic analysis reveal the presence of several infectious viruses in wastewaters

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National institute Of Biology

Aquatic environments represent a potential pathway for human, animal and plant virus transmission, which is especially relevant in the case of recycled water use for irrigation purposes. We analysed samples of the influent and effluent of wastewater treatment plant in Slovenia. Water samples were first concentrated using Convective Interaction Media (CIM) monolithic chromatography, a method that can efficiently concentrate viruses from high-volume water samples. Then, high throughput sequencing (HTS) was used to detect the presence of a wide array of viruses from different taxonomic groups. Using different bioinformatics tools, we defined the viromes of different samples in influents and effluents. We also studied the influence of different parameters, such as inclusion of a preamplification step, on the measured viral composition. Such approach allowed us to reveal high diversity of viral species in the analysed samples. Moreover, specific virus detection for the most common human and plant virus species was done by quantitative PCR and their integrity was confirmed using transmission electron microscopy. Because the stability and mechanical transmission of several plant viruses found by HTS is known to be high, we aimed to confirm their infectivity. Using test plants inoculated with concentrated samples of influents and effluents, we were able to confirm the infectivity of some plant viruses, such as *Pepper mild mottle virus* (PMMoV), *Tomato mosaic virus* (ToMV) and *Tobacco mild green mosaic virus* (TMGMV) from *Tobamovirus* genus (family *Virgaviridae*). Together, these data demonstrate that plant viruses remain infective even after conventional wastewater treatment. In the future, we will study methods that are able to inactivate viruses in water environments in a nature friendly and efficient manner, such as hydrodynamic cavitation.

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Development of continuous flow incubation system to detect single infective coliphage from a large volume water sample

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Introduction

In the field of health-related water microbiology, it is highly interested to develop a method to guarantee the absence of pathogenic/indicator microbes in water especially for the water that should be free from infectious risk such as drinking water. Then, absence of the microbes in a large volume of water should be proven rather than quantify their levels, as is seen in the standard value for *E.coli* in drinking water proposed by WHO, absence in 100 mL. To establish a method to ensure virological safety of drinking water is challenging because only a few particles of enteric virus can cause to indispensable health risk; it is supposed that concentration of rotavirus in drinking water should be below 1 particle per 100,000 L to achieve acceptable risk level. Log credit approach and bench scale test including spiking viruses or surrogates are applied to estimate treatment efficiencies and manage the quality of finished water, but the test of final product is also necessary in several conditions. For example, the log credit approach is not suitable in case of accidental change of the water quality. Therefore, monitoring of indigenous viruses in the treated water need to be taken into account.

In general, drinking water is treated by inactivation techniques such as chlorination, which allow a part of viral genes remain in the water. Then, infectivity assay including culture methods is favorable for viral detection rather than PCR-based assay. Coliphages, which are supposed to be the potential presence indicators for enteric viruses, seem to be a good target considering their superior growth rate.

Objectives

The aim of this study is to develop a method to detect infective virus/indicator in a large volume of water. A coliphage enrichment system was developed to detect one infective coliphages in a large volume of water. In this system, water sample and nutrient are continuously introduced into and drawn from a bottle where an *E.coli* strain is kept in the exponential growth phase like a fed-batch culture system. When a phage sensitive to the *E.coli* strain intruded into the system, the phage is expected to grow explosively. By testing a small amount of effluent from the system by the plate spotting test, presence/absence of the phage can be determined easily.

Methods

A phage enrichment system consists of a culture bottle (200 mL) and subsequent culture tube and retainment bottle (200 mL) as shown in the figure. In this system, sample containing nutrient (LB) was introduced into the culture bottle and drawn from the retainment bottle by a rotary pump. Hydraulic retention times in the culture bottle, the culture tube, and the retainment bottle were set to be two hours each. The culture bottle and culture tube were kept at 40°C. At first, LB broth (×5 dilution) and antibiotics were introduced into the system to grow *E.coli* (CB390 strain) in the culture bottle to reach the exponential growth and sufficient concentration. Subsequently, dechlorinated tap water spiked and mixed with coliphage MS2 and LB broth (×5 dilution) containing antibiotics, respectively, were introduced into the system. The samples from the culture bottle, the culture tube, and the retainment bottle were collected every two hours and subjected to MS2 determination by spotting onto a host bacterial lawn or plaque assay.

Results

Three trials for identification of the low concentration of infective MS2 have been conducted. In each test, concentration of MS2 in the sample (5 L) was set as 10 PFU/5 L. In this condition, the system is expected to be intruded by MS2 once per 5 hours and to retain one MS2 in the culture bottle for 2 hours. In every three trials, sample drawn from the culture bottle became positive for MS2 by plaque assay using 1 mL (around 10 PFU/mL) 4 or 6 hours after the sample introduction. After that, sample drawn from every component (5 uL) became positive for MS2 by the spotting assay, indicating that MS2 were sufficiently enriched in the culture bottle.

Conclusions

Considering that the first positive results were obtained within 6 hours, it is supposed that the first MS2 particle intruded in the system successfully started growth in the culture bottle within its retention time. After that, MS2 concentration in the culture bottle increases to be sufficient for detection by spotting. These imply that the method can identify 1 PFU/test volume of coliphage. MS2 concentration in this study was 2 PFU/L. By increasing test volume (pumping time), far lower concentration of coliphage may be identified.

Application of nanopore MinION for rapid viral pathogen detection in aquatic environments

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Objectives

The objective of this study is to establish a pipeline which enables on-site rapid viral pathogen detection for the aquatic environments using Nanopore MinIONTM sequencing.

Methods

Pure bacteriophages including MS2 (single-stranded RNA), P22 (double-stranded DNA); and human virus Adenovirus (double-stranded DNA) were used to establish and optimize the pipeline. The necessity of PCR enrichment prior to MinIONTM sequencing was evaluated. For sample pretreatment, effect of (multiple) DNase treatment and RNase treatment was examined.

For MinIONTM protocol, the impact of DNA repair step was examined, and the amount of barcoded sample in the adapter ligation step was optimized. The What's In The Pot (WIMP) workflow on EPI2ME was used for species identification.

Results

PCR enrichment appeared to be a crucial step to obtain adequate genomic material required for MinIONTM protocol. Ligation Sequencing Kit 1D and Native Barcoding Kit 1D was selected as standard workflow, following 1D Native Barcoding Genomic DNA protocol (Version:NBE_9005_v103_revO_21Dec2018).
Mock community experiments indicated that the optional DNA repair step not only significantly increased the sequence yield but also improved the accuracy of viral composition identification. While barcodes successfully distinguished single virus samples, <1% of total sequences were attributed to barcodes not used in the experiment. A primary challenge lies in the high proportion of host sequences after PCR enrichment for dsDNA virus. Neither an extra round of DNase treatment nor RNase treatment effectively alleviated the issue, whereas the latter interfered the detection of ssRNA virus (MS2). Increasing the amount of barcoded samples (7× recommended) improved the sequencing performance (pore occupancy from 7% to >50%).

Conclusions

With the appropriate protocol, MinION could be used as an efficient tool for rapid virus detection. Further optimization is needed for its application on viral community analysis.

Development of a Real-Time Cell Analysis (RTCA) method as a fast and accurate method for detecting infectious particles of hepatitis A virus

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Hepatitis A virus (HAV) is one of the most common agents causing acute liver disease worldwide. HAV has been increasingly reported as the cause of foodborne disease outbreaks. To date, a standard method is currently available for the detection of HAV genomes in foodstuffs by RT-qPCR (ISO 15216). Despite its usefulness in the investigation of foodborne viruses, this molecular method is unable to discriminate between non-infectious and infectious virus. Recently, an innovative Real-Time Cell Analysis (RTCA) technology based on cellular impedance measurements has been used to monitor the cytopathic effects (CPE) induced by several viruses.

The aim of this study was to evaluate cell impedance as a measure of HAV-induced CPE.

The cellular impedance of FRhK-4 cells infected or not with the adapted strain of HAV (HM175/18f) was measured in real-time using the xCELLigence system. Kinetics of cell impedance showed that HAV induced a decrease in cell index (CI) whilst CI remained constant in mock-infected cells. The HAV-induced CI drop was correlated with the onset of HAV-induced cell death as shown by using a cell viability assay and appeared earlier than visual observation under a microscope.

FRhK-4 cells were further infected with serial ten-fold dilutions of HAV. The kinetic curves showed that the CI drop in HAV-infected cells was delayed with the dilution of viral inoculum and that the sensitivity of the RTCA assay was 10^1 PFU per well. In addition, an inverse linear relation was established over a range of 5 $\log_{10}$ between the concentration of HAV and the time to reach 50% of CI decrease (TCI_{50}).

The innovative RTCA-based titration method for HAV could be used for high-throughput screening of inactivation treatments or antiviral molecules against HAV. Therefore this innovative method will be helpful in managing the viral risk in food virology.

Evaluation of a modified direct lysis method for detection of foodborne viruses on various fruit and vegetable

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Consumption of soft fruit and lettuce contaminated with noroviruses (NoV) or hepatitis A virus (HAV) frequently cause disease outbreaks. To ease surveillance and control viruses in these products and their supply chains, current detection methods need increased simplicity and sensitivity.

Towards a reference virus elusion-concentration method A, ISO 15216, a modified direct-lysis method B, requiring less time to perform, was evaluated for efficiency to recover genomes of spiked modelviruses, murine norovirus (MNV) and mengovirus (MC_0), and to eliminate RT-qPCR inhibitors during NoV and HAV detection from samples of 11 types of frozen fruit and vegetable of which some were naturally contaminated with NoV and HAV.

After processing samples of raspberries, strawberries, blueberries, blackberries, mango, spinach, dates and four different fruit mixes, the overall mean percentage of extraction efficiency of MNV and MC_0 were 6±14 and 5±6, respectively, using method A (n=33) and 68±70 and 42±35, respectively, using B (n=75). The mean percentage of RT-qPCR inhibition during detection of NoV genogroup I and II, and HAV were 5±48, 37±46 and 13±71, respectively, using method A (n=33) and -7±36, -8±25 and 2±28, respectively, using method B (n=75). Samples suspected to be implicated in viral disease outbreaks of gastroenteritis or hepatitis were also tested for the presence of NoV and HAV, which resulted in 2 of 20 and 4 of 42 positive samples using method A or B, respectively.

Method B resulted in overall better recovery rates in extracts from all sample types and less co-concentration of RT-qPCR-inhibitors in all sample extracts but dates and one fruit-mix, while taking less time to apply. We believe that method B has good potential for use in surveillance and outbreak investigations.

Targeted Amplicon Deep Sequencing Analysis: Diversity of adenovirus, papillomavirus and enterovirus in raw sewage

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Purpose/Objectives

Current protocols used for the detection of viruses in food/water give indication only on the specific pathogens, but will not provide information on other pathogens or variants.
In this study, Deep Amplicon Sequencing has been applied to evaluate DNA and RNA of emergent viruses in sewage. Enteroviruses (EV) presence and diversity were evaluated during an important outbreak in Catalonia causing neurologic affection to children. Also, human papillomaviruses (HPV), recently described to occur in water and potentially carcinogenic, were studied. Human adenoviruses (HAdV) were investigated as fecal indicators always present in urban sewage.

Materials/Methods

Viral particles present in sewage were concentrated by ultracentrifugation during a monthly sampling from April 2016 to March 2017. Viral concentrates were pooled per season and nucleic acids were extracted. The presence of different viral pathogens was assessed by nested (RT)-PCR. Amplicons produced were deep sequenced using Illumina Miseq 2x300bp.

Results

Around 45 EV species were detected. Interestingly, EV A71 with sequences highly similar to the C1 variants related to the outbreak were observed during this period of high incidence of clinical cases. In addition, a high variety of species resulted from the HPV amplification, being the *Alphapapillomavirus* HPV 6 and 66, described previously as oncogenic with low and high risk respectively, the more abundant types found. HAdV 40, 41 and 31, associated to gastroenteritis, were the HAdV species producing more reads in the urban sewage studied.

Conclusions

In this study, Deep Amplicon Sequencing protocols have been developed for surveillance purposes pointing all viral variants within a given viral family in a single assay. This study supports the idea that sewage can be used for surveillance, delivering additional information for early warning related to circulating emerging strains and outbreak tracing and investigation.

Viral processing of spiked human norovirus GI and GII influences viral persistence during disinfection of water

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Purpose/Objectives

Noroviruses cause significant global health burdens and waterborne transmission is a known exposure pathway. Chlorination is the most common method of disinfection for water and wastewater worldwide. The purpose of this study was to investigate the underlying causes for discrepancies in human norovirus (hNoV) resistance to free chlorine that have been previously published, and to assess hNoV GI and GII persistence during disinfection of municipal secondary wastewater (WW) effluent.

Materials/Methods

GI and GII stool samples were processed via Vertrel XF extraction, 0.45micron filtration, and sucrose cushion purification or 100K dalton ultrafiltration. At each step, portions of the hNoV samples were spiked into phosphate buffer or WW. Residual disinfectant and viral RNA degradation were analyzed over time.

Results

The choice of hNoV purification methodology prior to seeding viruses into an experimental water matrix influences disinfection outcomes. hNoV purification using solvent extraction and filtration were ineffective in removing organics resulting in an additional 190 mg/L and 440 mg/L as Cl2 of 15-second and 30-min chlorine demand, respectively, due to seeding noro-virus-positive stool at 1% w/v. These high organic loads impact experimental water chemistry and bias estimations of hNoV persistence. Advanced purification using sucrose cushion ultracentrifugation and ultrafiltration reduced 15-second chlorine demands by 99% and TOC by 93%.

Conclusions

A suite five of kinetic inactivation models were fit to viral reverse transcription-qPCR reduction data. Predicted disinfectant concentration x time (CT) values for 1, 2, and 3 log10 reduction of hNoV GI and GII in WW by free chlorine were 0.3, 2.1, and 7.8 mg-min/L, and 0.4, 2.0, and 7.0 mg-min/L respectively. These results indicate that conventional free chlorine disinfection is likely protective for public health with regards to noroviruses and will achieve at least 3-log reduction of hNoV GI and GII RNA despite previous reports of high hNoV resistance.

Specific detection of negative-strand viral RNA for determining norovirus infectivity

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Purpose/Objectives

In vitro cultivation of norovirus using stem-cell derived enteroids has been reported recently, which potentially allows determination of norovirus infectivity. The genome of norovirus is a positive-sense single-stranded RNA, which acts as a template for genome replication via the synthesis of a negative-strand RNA intermediate. The synthesis of negative-strand RNA serves as a marker for the active replication of norovirus. In this study, we aimed to develop a negative strand-specific RT-qPCR for determination of norovirus infectivity, using murine norovirus (MNV) as a model.

Materials/Methods

RAW 264.7 cells were infected with MNV, and viral RNA was extracted from cell pellets and supernatants. The number of negative-strand RNA was quantified using strand-specific RT-qPCR. Synthetic positive- and negative-strand RNA molecules were used to validate strand specificity of the RT-qPCR.

Results

Strand-specific quantification was achieved using specific RT primers tagged with a non-viral sequence at the 5' end, and no non-specific reaction with uninfected cells was observed. The numbers of negative-strand RNA in both cell pellets and supernatants increased exponentially during replication of MNV in host cells. Detectable increase in copy numbers of negative-strand RNA was observed before the development of CPE, suggesting the possibility of fast and sensitive viral infectivity determination using the proposed strand-specific RT-qPCR method.

Conclusions

We developed a negative-strand-specific RT-qPCR assay for rapid and accurate determination of MNV infectivity. This method is highly sensitive, likely because a number of negative-strand RNA can be produced from one infectious virion during *in vitro* replication. This approach could be applied to *in vitro* infectivity assay for human norovirus, which potentially contributes to elucidating the behavior of infectious norovirus particles, including occurrence in environmental samples, environmental persistence, and susceptibility to disinfectants.

KEYNOTE SPEAKER

Geographic distribution of cross-assembly phage in sewage

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Introduction/Objectives

Cross-assembly phage (crAssphage) was recently discovered to be highly abundant in the human gut and was subsequently proposed as a viral water quality marker. An unresolved question regarding the suitability of crAssphage for this application is its distribution within sewage worldwide. Currently, studies of crAssphage in human sewage have been largely limited to the United States and Europe (while other studies are in progress). In this presentation we will discuss metagenomic and qPCR investigations of the geographic distribution of crAssphage.

Methods

For metagenomic evaluation, previously published sequence data from sewage was aligned with crAssphage related contig sequences to estimate crAssphage presence and distribution in different geographic locals.
For qPCR evaluation, wastewater samples were collected from multiple counties and analyzed using qPCR for a previously published crAssphage assay, as well as other potential molecular and viral indicators, e.g. adenovirus and human polyomavirus.

Results

Metagenomic evaluation of the distribution of different genotypes in sewage is ongoing. Initial metagenomic evaluation and recent publications suggests different crAssphage genotypes dominate in different regions.
qPCR analysis of crAssphage demonstrates high detections (>95%) and concentrations in untreated sewage (averages $>10^6$ GC/100mL) in both Italy and the US. Initial evaluation shows that crAssphage concentrations are higher in samples that are positive for viral pathogens (e.g. HepE (p=0.02) and Bocavirus (p=0.03) in the Italian samples). Analysis is ongoing including samples from additional countries.

Conclusions

CrAssphage appears to be globally abundant in sewage and to co-occur with viral pathogens in sewage. Metagenomic evidence suggests the potential to optimize targets and assays for different geographic regions.

Will we ever be able to agree a single dose response model for Norovirus or is such a model merely a myth.

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Introduction

In 2016, van Abel and colleagues published a review of proposed dose response models for Norovirus of which she was able to identify 10 such models.[1] Although these different models all predicted about the same infection risk at exposures of $>10^3$ gene copies, some gave much higher risks than others at lower dose exposures, likely to be associated with food and waterborne exposure. As Van Abel pointed out all published dose-response models at that time were on GI and not on G11 genogroups.

Objectives

To consider whether or not a single dose-response model for Norovirus is feasible and if it is to suggest a strategy for developing such a model.

Methods

We develop a simple conceptual model of the relationship between population herd immunity and the dose-response model for Norovirus, especially in relation to the emergence of new escape mutants. We then undertake a structured review of the literature to validate and parameterise that conceptual model.

Results

When looking at the epidemiology of Norovirus infections several characteristics stand out. Firstly, there is substantial variation is reported episodes of infection from year to year. Indeed since 1996 there have been 6 pandemics of the virus.[2] Each pandemic seems to be associated with the emergence of a new strain through mutation. Indeed Noroviruses, like many RNA viruses are prone to hyper-mutation, especially in areas of the genome associated with key epitopes.[3] There is only weak cross-immunity between the different genogroups and even between different strains within the same genogroup.[4] The epidemiology of Norovirus infections can thus be thought of as a race between host population immunity and the emergence of new genotypes able to overcome the population immunity. Pandemics of Norovirus are then due to the appearance of escape mutants in much the same way that influenza pandemics follow antigenic drift and shift.

Secondly, calculated reproductive ratios also vary from one setting to another. Gaythorpe and colleagues reviewed the reproductive ratios for Norovirus reported in the literature and found that most reports gave a figure for R0 of about 2.0.[5] However, several studies estimated this to be much higher. Such variation in R0 would be consistent with the escape mutation hypothesis and so newer pandemic strains would likely have higher R0 values.

Finally studies on duration of immunity seem to give conflicting conclusions.[6] Whilst some human challenge studies have shown re-infection with the same strain after just six months other have suggested genotype immunity lasting up to 7 years. It is notable that the studies indicating the shorter duration of immunity used higher doses of virus[6] This would suggest that acquired immunity to Norovirus is not a binary phenomenon but rather a gradual one with immunity being able to be overcome by lower and lower doses as time passes after the initial infection.

We report a simple SIR model where unlike most such models immunity is not binary. We show that for any new escape mutant infection spreads rapidly throughout the population. The simple conceptual model assumes no cross-immunity between strains within a single host so probability of infection and/or illness and

is based on time since last infection and some innate infectivity of the virus in a totally immunologically naïve individual. We assume that immunity decays exponentially. Within this model initial results suggest that indeed our hypothesis that the dose response model varies with time even for the same strain is indeed correct.

Conclusions

Turning back to the issue of the dose response model for Norovirus, the above evidence would suggest that there cannot be a single model for all strains of the virus. Rather susceptibility to infection needs to be considered to a balance of the exposure dose and any pre-existing immunity whether caused by that or a related strain. In other words, in recent escape mutants the dose-response model is left shifted compared to strains that have been established in the population over past years. Recent escape mutants are likely to be far more infectious than strains that have been circulating for several years. So the dose-response model may vary from one strain to another and even within the same strain from one year to another. Whilst the use of outbreak data to generate dose-response models may offer a way forward such models are likely to over-estimate infectivity of most strains of the virus given that many outbreaks will be with the more recent escape mutant strain. We will discuss possible strategies for dealing with this problem.

Addressing research gaps to transition norovirus cell culture assays from research to environmental applications

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Pacific Northwest National Laboratory

Purpose / Objectives

Significant advances have been made that are beginning to address human norovirus (HuNoV) cell culture. However, of the published literature to date, the widespread application of these new HuNoV cell culture methods have been limited and is virtually non-existent for environmental applications. The objective of this review is to synthesize key findings to propose hypothesis driven research paths to drive the advancement of these new methods into mainstream environmental applications.

Materials/Methods

A review of the latest two methods to cultivate HuNoV reveals two different paths. Jones et al., showed that HuNoV GII.4 Sydney can infect BJAB cells and that intestinal bacteria may promote this infection. Ettayebi et al., used human intestinal enteroids (HIEs) to successfully culture both GI and GII HuNoV isolates. Here, they demonstrated that 1) HuNoV has an affinity for small intestinal enteroids, 2) secretor status is important, 3) infectivity may be promoted by treatment with bile acids, and 4) longer term passage in the HIEs is possible. Despite these findings for B-cells and HIEs, the assays prove to be difficult, and the amount of active replication is limited to approximately 2-3 Log_{10}. As recently as 2018, Oka et al., demonstrated replication in long term Caco-2 cultures, but it was not reproducible between different batches of cells.

Results/Conclusions

Our group has reviewed a significant amount of published literature and re-evaluated our findings to suggest several paths of investigation for HuNoV infectivity assays. One track proposes treatment of Caco-2 cultures with growth and differentiating factors that would drive the population of differentiated cells towards a small intestinal lineage. A second track would investigate co-culture of HIEs with B-cells to determine if HuNoV infectivity requires both cell types.

Assessment of viral pathogens, their indicators, and the applicability of quantitative microbial risk assessment in irrigation waters

Jennifer Pearce- Walker

University of Arizona

Purpose/Objectives

This project quantified and characterized viruses in irrigation water. First, southwest US irrigation waters were monitored for the occurrence of pathogenic and non-pathogenic viruses. Second, pathogenic viruses were evaluated for correlation with non-pathogenic viruses and current indicator organisms to assess indicators of viral contaminants. Finally, a quantitative microbial risk assessment (QMRA) was performed to understand the potential risk posed by viruses in irrigation water and the strengths and limitations of QMRA in irrigation water and food safety.

Materials/Methods

Pepper mild mottle virus, Aichivirus, bovine polyomavirus, and bovine adenovirus were evaluated as potential indicators of fecal contamination in irrigation waters and for their appropriateness relative to the traditional bacterial indicator, *E. coli*. Pearson correlation analyses were performed between established and potential indicator organisms and viral and bacterial pathogens.

Results

No significant correlations were observed between indicators or pathogens. A QMRA is being developed to predict health outcomes associated with consumption of fresh lettuce using viral pathogen concentrations from this study. Initial results indicate high potential risk of gastrointestinal illness following consumption of contaminated produce. These initial results were based on data collected during objective 1 and will be validated using virus data from irrigation water studies published in peer reviewed literature.

Conclusions

The lack of correlation between fecal indicators and viruses indicates intentional safety caps and using *E. coli* as an indicator of water quality. While initial QMRA results indicate high potential risk of gastrointestinal illness, the significant number of non-detects in samples hinder fully understanding and modeling health risks. Improved understanding of the appropriateness of fecal indicators in irrigation water and the risk posed by exposure to irrigation water via fresh produce can inform management decisions, improving food crop safety.

Health risks associated with playgrounds: Decay of human enteroviruses in natural sand and fine gravel

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Objectives

Sandboxes and playgrounds are commonly used by children all over the world. Currently little to no legislation is available for the detection of pathogens in sand used in playground and while beach sands are increasingly being studied as a reservoir of potential pathogens, less is known about the occurrence of such microorganisms in other recreational sands. In this study, we intended to determine how viruses behave and decay when present in these health-sensitive locations.

Methods

Human enterovirus (EntV) was inoculated in two distinct types of sand, natural sand and fine gravel. Samples were incubated under direct sunlight and in the shadow and analyzed over the period of one month to determine viral inactivation under these conditions. Mengovirus (vMC_0) was used as a process control for the elution and concentration of viruses from sand. Viruses were eluted and filtered through a membrane for concentration and determined by RT-qPCR.

Results

The recoveries using vMC_0 varied from 7 to 22% (mean 12%) for natural sand and from 6 to 30% (mean 12%) for fine gravel. Inactivation of EntV in natural sand incubated under direct sunlight was more than 2log (2.34log). For fine gravel, the inactivation was determined to be around 2log (2.06log). No perceivable decrease in EntV numbers was determined either in sand and gravel that when incubated in the shadow. Inactivation in gravel was slower that in sand, with the first showing the first real decrease only after 11 days of incubation and the second displaying inactivation only after 4 days of incubation.

Conclusions

Playgrounds and sandboxes are usually located close to trees and are therefore generally in the shadow, the results presented in this study show that having either contaminated natural sand or gravel may pose a great risk of infection to children.

Effectiveness of sodium deoxycholate pre-treatment to improve viability RT-qPCR for discrimination of inactivated viruses in drinking water

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Objectives

The aim of this study was to evaluate ability of sodium deoxycholate (SD) to improve the penetration of ethidium monoazide (EMA), propidium monoazide (PMA) or cis-dichlorodiammineplatinum (CDDP) into damaged viruses and so minimize the false-positive RT-qPCR signal. Moreover, the applicability of EMA/PMA/CDDP-RT-qPCR with SD pre-treatment was tested under the presence of environmental matrices from actual drinking water concentrates.

Methods

To optimize the SD concentration, the heat-inactivated Aichi virus (AiV) (at 90^{o}C for 1 min) was pre-treated with various concentrations of SD (0.01-0.5%), then treated with EMA (100 µM), PMA (100 µM) or CDDP (1,000 µM) and followed by RT-qPCR. Besides, EMA/PMA/CDDP-RT-qPCR pre-treated with optimal SD concentration was used to evaluate the inactivation of AiV by chlorine disinfection (1mg/L chlorine and 1-10 min contact time). Finally, their performance was tested on a total of six drinking water concentrates (500 L) with spiked the chlorine-treated AiV (1mg/L chlorine and 5 min contact time).

Results

EMA/PMA/CDDP-RT-qPCR with SD pre-treatment at 0.1% showed a greater $\log_{10}$ reduction of heated AiV than those pre-treated with other SD concentrations. Thus, the concentration of 0.1% was considered optimum for improving the treatment of EMA/PMA/CDDP prior to RT-qPCR. In addition, EMA/PMA/CDDP-RT-qPCR with 0.1% SD pre-treatment found to be more effective than those without SD pre-treatment in the discrimination between infectious and chlorinated AiV at different contact times, while CDDP-RT-qPCR with 0.1% SD pre-treatment was the most effective. All drinking water concentrates did not interfere with the performance of EMA/PMA/CDDP-RT-qPCR with 0.1% SD pre-treatment.

Conclusion

SD pre-treatment at 0.1% was able to effectively improve the treatment of EMA/PMA/CDDP and therefore can be used with EMA/PMA/CDDP-RT-qPCR for more accurate quantification of potential infectious viruses in drinking water.

Surveillance of enteric viruses in sewage reveals high viral diversity and synchronous time patterns with number of hospital patients infected with the viruses identified

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Purpose/Objectives

Sewage harbors diverse human pathogens, including viruses, and serves as an efficient vehicle to reach new hosts. However, little is known about the prevalence and diversity of enteric viruses in sewage and the possibility of predicting outbreaks by relating the type and quantification of viruses identified in wastewater with the number and time of hospitalized patients infected with these pathogens.

Materials/Methods

Fourteen common enteric viruses were monitored by real-time-PCR in influent from two Swedish sewage treatment plants, at Knivsta and Gothenburg. The influent was sampled weekly during December 2016 in Knivsta and biweekly during 2017 in Gothenburg. The viral diversity in influents at Knivsta was analyzed by metagenomics sequencing. The number of estimated patients infected with identified enteric virus was calculated based on viral genomes in wastewater and compared with the number of hospitalized patients within Gothenburg during 2017.

Results

Seven and eleven common enteric viruses were detected in influents at Knivsta and Gothenburg. Metagenomics sequencing of influents at Knivsta identified 327 viral species belonging to 25 known families. Viruses from five viral families are associated with human diseases. One-year monitoring of wastewater in Gothenburg showed that norovirus GII was at high levels throughout the year. The comparison between estimated infected persons and hospitalized patients showed a synchronous time pattern in winter when peaks of viral genomes in sewage were 4-6 weeks earlier than the peaks of the number of hospitalized patients.

Conclusions

These results demonstrate a high viral diversity including some common enteric viruses in sewage in Sweden. The synchronous time patterns but several weeks difference between the peaks of viral amount in sewage and hospitalized patients shows this technique can provide the basis for early warning of virus outbreaks. Continuous monitoring with detection and quantification of enteric viruses in sewage should be considered in the future.

KEYNOTE SPEAKER

The role of virus structure in removal and inactivation of health-related viruses

Brooke Mayer

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Introduction

Virus structure is the defining feature of virus stability, fate, and transport. The nucleic and amino acid composition of each individual food and environmentally relevant virus denotes its behaviour with respect to its fate and transport, both in the environment and in built systems.

Objectives

The primary objective of this conference presentation is to discuss the state of the art with respect to current research focused on the role of virus structure in stability, fate, and transport studies. Specific emphasis will be allocated to discussing the role of virus structure in physical removal (e.g., isoelectric point and size) versus virus inactivation (e.g., nucleic vs. amino acid composition).

Methods

A combination of methods, both cultural and molecular, will be discussed as relevant to elucidating the fate of food and environmentally relevant viruses. Specific mention of virus removal and inactivation using water treatment techniques such as electrocoagulation and UV irradiation will be made.

Results

Results from different research groups will be highlighted in this presentation, including studies of proteomics (Wigginton et al.), inactivation (Mayer et al.), and physical removal. While chemical oxidizing disinfectants tend to target nucleic acids, alternative disinfectants such as UV may better target amino acids, thereby enhancing virus inactivation.

Conclusions

Virus structure, in terms of genomics, proteomics, as well as physical structure play an important role in virus behavior in the environment, which in turn dictates human and environmental safety.

Structural characterization of thermoresistant enteroviruses

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Introduction

Human enteroviruses are a major cause of waterborne disease outbreak worldwide. Their environmental persistence is an important factor determining their dissemination and the risk of environment-to-human transmission. Recently, we determined that different serotypes and strains of enterovirus (Coxsackievirus B1, B4, B5 and Echovirus 11) differ in their susceptibility to disinfectants and environmental stressors. Here we further explore the susceptibility of these viruses to thermal inactivation, an important determinant of environmental persistence.

Objectives

The goal of this study is:

1) To identify the mechanism of virus inactivation during thermal inactivation,
2) To characterize structural features that enhance thermal stability.
3) To identify mutations associated with thermal stability.

Methods

Enteroviruses were either obtained commercially or were isolated from different wastewater treatment plant influents. Their genomes were sequenced and their capsid 3D structures were built by homology-modelling using the crystal structure of Coxsackievirus B3 as template. Their thermal resistance, quantified as the inactivation rate constant at 55°C, was correlated to the capsid melting temperature and to various structural features of the viral capsid. Findings regarding the influence of structural features on thermal stability were validated by comparing the corresponding features in native and thermoresistant coxsackievirus B5 produced by experimental evolution.

Results

Echovirus 11 and CVB4 were more sensitive to thermal inactivation compared to serotypes CVB5 and CVB1. The melting temperatures correlated to the thermal inactivation rate, indicating that the primary reason of infectivity loss was the disruption of the viral capsid. Structural analysis revealed that thermal stability was associated with stronger electrostatic interactions between the capsid subunits. Finally, these findings were confirmed in heat-adapted viruses, where thermal stability was caused by few specific mutations in the VP1 protein that also enhanced electrostatic interactions between capsid subunits.

Conclusions

Electrostatic interactions between the virus capsid subunits are a major factor determining thermal stability of enteroviruses, and this trait can be readily acquired through few specific mutations in the viral proteins. This feature appeared to be similar in all strains of a given serotypes.

Physisorption and chemisorption of T4 bacteriophages on amino functionalized silica particles

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Arizona State University

Objective

Bacteriophages, or phages, are receiving increasing interest as recognition tools for the design of bioactive surfaces. However, to maintain the activity of surface-bound phages, the immobilization strategy must provide the right orientation and not compromise phages' integrity. The objectives of this study were to characterize the phage sorption capacity and the immobilized phage activity for aminated silica particles functionalized with T4 phages, in order to identify guidelines for the fabrication of colloidal phage carriers.

Materials and Methods

Two functionalization strategies were compared; physisorption, based on non-covalent electrostatic adhesion, and chemisorption, where a covalent amide bond is formed between the phage and the particle. Functionalized particles were characterized by electrophoretic mobility analysis and infrared spectroscopy. Phage-loading for each particle functionalization approach was investigated using plaque assays and flow cytometry.

Results

We report that chemisorption, at maximum adsorption conditions on 1 µm particles, has 16 functional phage per particle, 2.5 times more than by the physisorption method. Particle diameter is shown to have an important impact on phage attachment and 1.8 µm particles were found to have ~4 times more phages per surface area than 0.5 µm particles. Higher surface coverage is attributed to the lower steric hindrance on bigger particles.

Conclusions

These findings provide important guidelines for the design of phage-functionalized particles for environmental, biomedical, or sensing applications. Higher surface phage density can be obtained using larger particles and chemisorption methods.

Interaction between human noroviruses and *Acanthamoeba castellanii*: influence on the viral persistence in water environment

Benoit Prevost

CHU Grenoble / Abiolab Asposan

Although clinical epidemiology lists human enteric viruses among the primary causes of acute gastroenteritis in human population, their circulation and persistence in water environment remains poorly investigated. These viruses are excreted by the human population into the sewers and may be released in rivers through the effluents of wastewater treatment plants. A broad spectrum of particles and microorganisms are present and so susceptible to interact with enteric viruses. Among these enteric viruses, human noroviruses (HuNoV) are one of the leading causes of waterborne gastroenteritis worldwide. Free-living amoebae (FLA) are already identified to interact with different microorganisms and are frequently detected in water environment. Consequently, FLA could potentially play a role of reservoir of HuNoV and so facilitate the environmental transmission and persistence of HuNoV. The main objectives of this study were on the one hand to evaluate interactions between different noroviruses (NoV GI, NoV GII, and MNV-1) and *Acanthamoeba castellanii* and, on the other hand, to assess the protective effect potentially provided by amoebae against disinfection treatments such as heat shock, chlorination and ultraviolet radiation. The presence and persistence of noroviruses with and without amoebae were evaluated by molecular biology methods, culture methods, and electron microscopy. Whatever the treatment, viral genomes and infectivity reduction rates were significantly decreased from 1 to 3 log10 when noroviruses were in presence of *Acanthamoeba castellanii*. In conclusion, these results revealed that FLA can contain noroviruses and so provide them a protection against several disinfection treatments frequently implemented in drinking water plants. Therefore, enteric viruses could be more resistant in water environment due to the presence of microorganisms and particles and so could explain some waterborne gastroenteritis by consumption of tap water.

Enterovirus interaction with organic matter: Impact on virus stability

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Introduction

As the supply of safe drinking water is essential for ensuring the health of populations, water produced from polluted resources goes through various disinfection barriers in drinking water treatment plants. Nonetheless, the occurrence of outbreaks linked to water consumption suggests that virus removal is sometimes overestimated. In the environment, viruses encounter many different compounds which can impact their state (eg. free, aggregated) and their stability. Indeed our early results have shown that virus aggregation and interactions with organic compounds may impair disinfection treatments efficiency. It also recently emerged that human enteric virus interact with gut bacteria, and that bacterial cell wall compounds play a significant role in these interactions. These interactions are also driven by the capsid protein sequence. Several clues indicate that different mechanisms are probably involved in the processus of viral protection against disinfection treatments. They still need to be identified in order to fully apprehend the different factors that promote virus stability in drinking water.

Objectives

The aim of this study was to identify environmental bacterial populations and soluble organic compounds that can increase virus stability. We investigated whether bacterial communities, environmental particulate matter originating from surface waters, or organic soluble compounds can impact virus resistance to heat treatment.

Methods

We first studied whether enteroviruses could interact directly with bacterial cell compounds. Transmission electron microscopy was performed to visualize the spatial aggregation of viral particle structure in the presence of bacterial lipopolysaccharides and peptidoglycans.
Water samples from the Seine River were collected and fractionated by sequential centrifugation and ultrafiltration. Several particulate fractions were obtained and their impact on coxsackievirus B5 (CV-B5) stability was investigated. To this end, CV-B5 was incubated with the different fractions for 2 hours and heat treated for 1h at 50°C. The remaining infectivity was evaluated by end point dilution assay on BGMK cells. Simultaneously, bacterial populations present in the different fractions were identified based on 16S ribosomal DNA sequence by next generation sequencing. The bacterial candidates were isolated and individually tested to confirm or infirm their capacity to stabilize viruses.
Regarding soluble compounds we considered several carbohydrates, amino acids or proteins. We performed the same test as described above (incubation, heat treatment and infectivity measurement), and we also investigated their impact on capsid integrity using a Particle Stability Thermal Release Assay. In this assay, viruses were gradually heated in the presence of a nucleic acid dye that can reach the viral genome when the capsid loses its integrity. SyBR green II was used to monitor viral genome accessibility.

Results

We previously observed that viruses and polysaccharides co-incubation increased viral particle stability and resistance to oxidative treatment (chloration and ozonation). In this study, electron microscopy observations showed the colocalization of CV-B5 and lipopolysaccharides or peptidoglycans. This observation suggests that direct interactions could be implicated in the viral stability.

In order to better describe these interactions, various fractions originating from surface water were isolated. Some of them did not induce significant differences in the inactivation efficacy compared to controls, while other conferred to CV-B5 a significant resistance to heat.
Regarding soluble organic matter, various results of protection were observed, but the characterization of these compounds is still in process.
The analysis of bacterial diversity in the fractions that confer protection permitted to identify potential bacterial candidates that may interact with viruses and may confer some protection to the viruses. .

Conclusions

Improving our knowledge of the interactions in which enteric viruses are engaged is crucial to better understand their stability and fate. Virus aggregation and the impact on virus inactivation during water treatment is more and more documented, but interactions between viruses and other micro-organisms (or compounds derived from them) could largely modify what we know of virus elimination in water treatment.

KEYNOTE SPEAKER:

Inactivation and removal of viruses: is this a matter of virus evolution?

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Human enteric viruses are excreted in feces from infected individuals and included in wastewater. It is critical to remove/inactivate them in wastewater treatment processes before release to water environments. However, the high mutation rate of human enteric viruses raises concerns about the emergence of strains that cannot be effectively removed/inactivated in wastewater treatment plant. In our previous study, we proved that a murine norovirus population with lower susceptibility to free chlorine cam be acquired by the repeated exposure to the disinfectant. We also found a unique non synonymous mutation (from phenylalanine to serine) at nt 7280 in ORF3, encoding the minor capsid protein VP2, in chlorine-exposed populations. Plaque-purified clones with the corresponding mutation were acquired from the chlorine-exposed populations, and it was confirmed that all the mutants had lower susceptibility to free chlorine than the wild-type. These results indicate that a single mutation in VP2 affects the free chlorine susceptibility of murine norovirus. In another study, we demonstrated the genotype-dependent removal of human norovirus particles with a microfiltration (MF) membrane in the presence of bacteria bearing histo-blood group antigens (HBGAs). Three genotypes (GII.3, GII.4, and GII.6) of norovirus-like particles (NoVLPs) were mixed with three bacterial strains (*Enterobacter* sp. SENG-6, *Escherichia coli* O86:K61:B7, and *Staphylococcus epidermidis*), and the mixture was filtered with an MF membrane having a nominal pore size of 0.45 μm. Our results indicate that the location of HBGAs on bacterial cells is an important factor in determining the genotype-dependent removal efficiency of norovirus particles with the MF membrane. In this keynote speech, the genotype/strain dependent efficiency of inactivation and removal of enteric viruses is discussed based on the results from our studies and other literatures.

A review of the current state of UV virus inactivation

George Diefenthal

NeoTech Aqua Solutions, Inc.

UV technology has long been recognized as an effective means of inactivating microbes and viruses. Viruses, with their simpler structures pose some challenges as they have some effective repair mechanisms available. The discussion will review the inactivation methods of UV vs. other popular methods such as chlorine and ozone. We will also review the current knowledge and methods of addressing the repair mechanisms. This will be followed by a review of the various UV technologies on the market and how they perform. The discussion will end with a overview of the regulatory landscape is it stands today.

Virus inactivation by ozone in natural matrices: potential proxies for viral inactivation

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Introduction

Ozone is an efficient disinfectant for waterborne viruses. In a previous study, inactivation of a suite of human viruses and bacteriophages by ozone in buffer solutions was measured, and inactivation rate constants ($k_{O3\text{-}Virus}$) were determined on the order of 10^6 $M^{-1}s^{-1}$. These buffer solutions have a simple composition, whereas in natural waters, virus inactivation may be inhibited by different matrix constituents.
Furthermore, determining the ozone exposure (controlling factor for virus inactivation) in natural matrices is not practical. Then, this approach is not applicable to monitor virus inactivation in real treatment systems. Alternatively, inactivation may be monitored based on an "easy-to-measure" proxy. Proxies are an indirect measure of the ozone exposure, which in turn, depends on the specific ozone dose ($mgO_3/mgDOC$) used. Here, we explore if UV_{254} absorbance (ΔUV_{254}) and carbamazepine (CBZ) abatement, an ubiquitous micropolutants in wastewater, may serve as proxies for virus inactivation.

Objectives

(1) Determine the ozone exposures as a function of the ozone dose in surface (SW) and wastewater (WW)
(2) Investigate the influence of natural matrices on $k_{O3\text{-}Virus}$
(3) Validate potential proxies for virus inactivation during ozonation of SW and WW.

Methods

We determined the ozone exposure for different specific ozone doses in SW and WW. Then, MS2 and Coxsackievirus B5 (CVB5) inactivation were measured at different specific ozone doses, along with ΔUV_{254} and CBZ abatement.

Results

$k_{O3\text{-}Virus}$ in SW were equivalent (MS2) or slightly higher (CVB5) than in the clean buffer system. However, in WW both constants were slightly lower compared to buffer solutions. This indicates that the WW matrix constituents exert a protective effect on the viruses, however, this effect was not dramatic. Ozone remained an efficient disinfectant even in wastewater. In addition, we evaluated if ΔUV_{254} or CBZ abatement were well correlated with specific ozone dose and inactivation. Nevertheless, ΔUV_{254} is not practical in low DOC waters.

Conclusion

Ozone is an efficient disinfectant for virus inactivation in natural matrices. Furthermore, two potential proxies to assess virus inactivation were identified.

Inactivation mechanism of rotavirus, human norovirus, and human norovirus surrogate Tulane virus by food sanitizers

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University of Illinois at Urbana-Champaign

Foodborne diseases have been frequently reported worldwide, thus it is important to ensure food safety. Especially fresh produce eaten raw could be a carrier of pathogens to consumers. Viruses are the most common pathogens transmitted via food and estimated to be the largest cause of foodborne disease. Enteric viruses such as rotavirus (RV) and human norovirus (HuNoV) have been detected in various kinds of water sources and on fresh produce, thus fresh produce irrigated with water contaminated with those viruses could be a cause of foodborne illnesses. Although vegetable is commonly sanitized before being sold to customers, the inactivation mechanisms responsible for enteric virus inactivation are not fully understood. Therefore, the main focus of this study was to understand the inactivation mechanisms of RV and HuNoV, and HuNoV surrogate Tulane virus (TV) by identifying capsid damage and virus binding ability to cells. We determined inactivation efficacies and inactivation mechanisms of three kinds of vegetable sanitizers, chlorine, peracetic acid (PAA), and malic acid (MA) with thiamine dilauryl sulfate (TDS). An assay using porcine gastric mucin-conjugated magnetic beads (PGM-MBs), which contain RV, HuNoV, and TV receptors, was developed to identify whether these sanitizers compromised the binding of virus particles to host cell receptors. Our results demonstrated that TV was more resistant to the disinfectants above compared to RV strain OSU when exposed for 15 sec to 3 min to either 2.5 ppm free chlorine or 0.05 % malic acid mixed with 0.005 % TDS. Our binding assay identified capsid damage of chlorine treated RV and TV and less binding ability to receptors. In this presentation, we will provide comprehensive comparisons of RV, HuNoV, and TV inactivation mechanisms with three sanitizers.

A RTCA-based assay as an innovative approach for thermal inactivation studies of Hepatitis A virus (HAV)

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Hepatitis A virus (HAV) is mainly transmitted through the **fecal-oral route**, either directly by close contact with infected people or indirectly through ingestion of contaminated food and water. Controlling transmission through their removal from drinking water and foodstuff is an important challenge in reducing the burden of viral foodborne illnesses.

The aim of the study was (i) to evaluate the previously developed Real-Time Cell Analysis (RTCA) for thermal inactivation studies and (ii) to compare inactivation kinetic curves obtained with other available detection methods (Plaque Forming-Unit (PFU) assay) and molecular-based methods (dye-combined method (EMA-RT-qPCR) and RT-qPCR).

The impedance of FRhK-4 cells uninfected or infected with HAV suspensions heat-treated (37°C; 50°C; 56°C; 65°C; 72°C or 80°C) or not was measured in real-time using the xCELLigence system. In mock-infected cells, CI remained constant whereas a CI drop appeared in HAV-infected cells according to the temperature and time treatment. The times to reach 50% of CI decrease (TCI_{50}) were determined to quantify the remained infectious HAV following heat-treatment from a standard curve established between the concentration of HAV and the TCI_{50}. Increasing the temperature and/or the duration of heat treatment at constant temperature led to a delay in CI decrease until there were no longer any decreases in CI.

Inactivation kinetic curves obtained by using the RTCA assay followed the same profile than the ones obtained with the traditional PFU assay regardless the heat treatment. On the contrary, kinetics profiles obtained by using the EMA-RT-qPCR were close to the ones obtained with both cell-based methods only for high temperatures ($\geq$ 56°C).

As the RTCA-based titration method presents many advantages in comparison with the traditional PFU method (*i.e.* less fastidious, giving results faster, less expensive), it could be helpful to validate technological treatments used in food industries for a better viral risk management.

Impact of intermittent operation on virus removal by residential RO membrane

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[1]The University of Tokyo, Japan [2]Thuy Loi University, Hanoi, Vietnam

Objectives

The aims of this study are i) to assess the virus removal by residential RO membrane under intermittent operation by bench-scale experiment and ii) to evaluate the contribution of intermittent operation to the deterioration of the actual residential RO membranes in Hanoi, Vietnam.

Materials, Methods

i) Virgin RO membranes (Dow Filmtec, US) were operated intermittently for 10,000 cycles, which consisted of 10 seconds of pressurization by the pump (0.5 MPa) and 20 seconds of pump stop. The removals of spiked MS2 and φX-174 were evaluated in every 1,000 cycles. As a control, RO membranes were operated at constant pressure. Their removal efficiencies were also evaluated.
ii) A total of 27 used residential RO membranes (Dow Filmtec, US) were collected from local houses in Hanoi. They were divided into two groups based on the total pressurized times estimated by the information on membrane age and use frequency (group 1: <4000 times, group 2: >4000 times). The efficiencies of virus removal (spiked MS2 and φX-174) in the two groups were determined and statistically compared.

Results

i) The membrane operated at constant pressure showed a stable φX-174 log removal (4 log10).
However, in the intermittent operation, log removals of φX-174 were maintained at 4 log10 only for first 3,000 – 5,000 cycles, then dramatically dropped to 1.8 log10 after 10,000 cycles. It indicates that intermittent operation promotes the membrane deterioration resulting in the decrease of virus removal efficiency.
ii) The MS2 removals in group 2 (more pressurized) were significantly lower than those of group 1 (less pressurized). This result suggests that the intermittent operation may mainly contribute to the membrane deterioration in households.

Conclusion

Intermittent operation gave negative impact on the virus removal by residential RO membrane.
Therefore, pressurized times should be considered for maintaining the performance of virus removal.

Removal efficiency of indigenous F-specific RNA phages in inline coagulation and micro-filtration system affected by different coagulation characteristics

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Objectives

For better understanding of virus removal in the water treatment, log-removal values (LRVs)of variety of viruses should be investigated. The LRV of F-specific RNA phages (FRNAPH) by 0.2□mmembrane filtration with pre-coagulation under buffer condition was defined as a reference value of coagulation characteristics. Then, LRVs in inline coagulation and micro-filtration of various FRNAPHs using tertiary treated waste water were compared with the reference value. The effect of the raw water quality in removing phages was also studied.

Methods

Three strains, FRNAPH genotype I (GI) and GIII, were selected among 20 indigenous isolates based on the LRV under pH5 buffer conditioning bench scale. Tertiary treated wastewater (pH7.2, 0.7 NTU) were applied as the raw water, to which Polyaluminium chloride(PAX-XL19) was continuously injected at a dose of 50mg/L. The test water was continuously introduced to inline static mixer and filtration process by ceramic filter for 2weeks. FRNAPH was added in the test water to observe the removal efficiency. Phosphate buffer (pH7.2, 6.7mmol/L) was also used as the raw water.

Results

Average LRVs(n=4)of each selected indigenous strains by inline coagulation and micro-filtration were >7.30, 6.81, and >6.50 (GI, GI, GIII),among which only one GI strain was detected from the filtrate. This strain was the least coagulated among the three strains used in inline experiment. Its LRV was almost 0.2when phosphate buffer was used as the raw water.

Conclusions

LRVs of three strains were over 6.0 where one GI strain was detected in the filtrate of the inline experiment. The results showed that different coagulation characteristics of FRNAPHs were directly reflected in LRVs in inline coagulation and micro-filtration. LRVof the detected GI strain was only 0.2when using phosphate buffer. Therefore virus removal may be attributed to virus absorption to micro-flocsin the raw water in inline coagulation and micro-filtration.

Synergistic effects of TiO_2 and PAA for inactivation of viruses

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Effective and sustainable water treatment technologies are needed to ensure the microbial quality of treated water. Advanced oxidation processes (AOP) are prospective treatment systems for removal/mitigation of contaminants by using a combination of oxidizing agent and/or UV. The synergistic effect of titanium dioxide (TiO_2) or peracetic acid (PAA) with UV presents additional advantages for inactivation of pathogens in water. In this study, we tested different AOP systems comprising of TiO_2 and/or PAA in combination with UV. The TiO_2/UV system was tested in collimated beam and PhotoCAT; whereas PAA/UV system was tested in a NeoTech UV reactor.

The collimated beam apparatus contained a 15-watt, low-pressure mercury arc bulb. The PhotoCAT system is an integrated UV/ceramic membrane reactor, contained four 220-watt low-pressure mercury UV lamps. The NeoTech reactor contained a single high output 98-watt lamp.

The efficacy of TiO_2/ UV system is impacted by delivery configuration. Tendering of TiO_2/ UV under PhotoCAT and bench scale collimated beam yielded opposite trends in the inactivation of P22 bacteriophage. Higher TiO_2 doses resulted in a higher inactivation in the PhotoCAT and lower inactivation in the collimated beam. Adding 40 mg/L of TiO_2 in the photoCAT system improved P22 inactivation by 25% while it slightly decreased P22 inactivation in collimated beam apparatus. Furthermore, P22 inactivation using PAA/UV system contrasted with UV. Compared to UV, the P22 inactivation increased 18% and 70% using PAA/UV systems consisting 1 and 10 ppm PAA, respectively. However, at lower PAA concertation, no such differences in P22 inactivation was noticed.

Overall, the inactivation synergy of PAA/UV system was stronger (with a minimal pH change) for virus inactivation than that of TiO_2/UV system at the same chemical dose. The results of this study bridge the knowledge gaps on the effectiveness and applicability of UV technology in AOP systems for the inactivation of waterborne viruses.

KEYNOTE SPEAKER:

Successful replication of human norovirus in human intestinal enteroids as a model to measure virus inactivation

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Human noroviruses are the most common cause of epidemic gastroenteritis worldwide and a leading cause of foodborne illness in the US. Since the discovery of norovirus in 1972, many research groups have attempted to grow human norovirus, most of whom have failed or whose results were difficult to replicate in other laboratories. Recently, successful replication of human norovirus was reported using human intestinal enteroids (HIEs). In this study, we infected duplicate 96-wells of HIE monolayers with 80 human norovirus positive stool samples [12 genogroup (G) I, 65 GII and 3 GIV] from outbreaks and sporadic cases of acute gastroenteritis collected between 2000 and 2017. Successful replication was shown for six different norovirus GII genotypes (GII.1, GII.2, GII.3, GII.4, GII.14 and GII.17) including 3 different GII.4 variants. Identical levels of replication were obtained consistently for several human norovirus strains tested at several time points over a 1 year period. The 50% infectious dose was 2.1 x 10^3 genome copies/well for GII.4 Den Haag strain, 5.6 x 10^2 genome copies/well for GII.4 Sydney strain and 3.9 x 10^3 genome copies/well for GII.3 strain. Among the strains that successfully replicated, three GII.4 strains (GII.4 Den Haag, GII.4 New Orleans, GII.4 Sydney) were chosen to assess virus inactivation with increasing concentrations of chlorine (0 to 5000 ppm) and with 70% ethanol or isopropanol. Regardless of exposure time (1 or 5 min), alcohols slightly reduced, but did not completely inactivate, human norovirus replication. In contrast, complete inactivation of the three GII.4 viruses occurred at concentrations as low as 50 ppm of chlorine. Taken together, our data confirm the successful replication of human noroviruses in HIEs and their utility as tools to study norovirus inactivation strategies.

Two drinking water outbreaks caused by sapovirus in Finland

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National Institute for Health and Welfare

Several factors may cause drinking water outbreaks, including raw water contamination, treatment deficiencies and distribution network failure. This study describes two drinking water outbreaks in Finland in 2016 and 2018. Both outbreaks caused approximately 450 illness cases, and were initially caused by the pipe breakage and subsequent wastewater intrusion into distribution system. Patient samples were analysed for enteric viruses (noro-, sapo-, astro-, rota- and adenoviruses), pathogenic bacteria (salmonella, shigella, yersinia and campylobacter, and in the outbreak II pathogenic *E. coli*) and parasites (*Dientamoeba fragilis*, *Cryptosporidium, Giardia lamblia* and *Entamoeba histolytica*). The analysed microbes for water samples were selected based on the results of patient samples. In addition, water quality indicator microbes and host-specific source tracking (MST) markers were analysed. Sapoviruses were detected in the patient samples collected from both outbreaks. Also, adenoviruses and *D. fragilis* (outbreak I), and noroviruses, astroviruses and ETEC (outbreak II) were detected from the patient samples. Sapovirus was detected from a drinking water sample in outbreak II. Further, wastewater samples taken from the contamination sources contained sapoviruses in both outbreaks. The MST markers proved useful in the detection of contamination and were in concordance with pathogen findings. Boil water advisory was set in both outbreaks and alternative drinking water sources were organized to restrict the outbreaks. Chlorination was efficient measure to clean the contaminated distribution network. This study highlights the emerging role of sapoviruses as a waterborne pathogen, and warrants the need for testing of multiple viruses during outbreak investigation. Further, *D. fragilis* caused concern in outbreak I. The ageing water infrastructure poses a major challenge for water supply and sewerage services, and may compromise drinking water safety even more often in the future.

Enhanced poliovirus detection in Pakistan environmental samples using the bag-mediated filtration system

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Purpose/Objectives

Poliovirus (PV) environmental surveillance plays an important role in ensuring the completeness of the global eradication program. The bag-mediated filtration system (BMFS) was developed to enhance surveillance sensitivity. This study compared BMFS environmental surveillance results with the method recommended by the World Health Organization (WHO; two-phase separation). This study provided a unique opportunity to monitor the disappearance of Sabin-like PV type 2 (SL2) from the environment after global cessation of SL2 use in routine or supplementary immunization activities in April 2016.

Materials/Methods

From February 2016-present, BMFS (5.8±0.1-L) and two-phase (500-mL) samples were collected concurrently from 15 sites in 12 Pakistani districts (n=295), and analyzed for Sabin-like PV (SL), wild PV (WPV), and vaccine-derived PV (VDPV) types 1, 2, and 3 by the WHO algorithm.

Results

PV was detected in a majority of BMFS (83.7%) and two-phase samples (64.1%). Compared to two-phase samples, BMFS resulted in more frequent detection of SL1 (p<0.0001), SL2 (p=0.035), SL3 (p<0.0001), and WPV1 (p=0.0081). The BMFS detected VDPV2 during one and WPV1 during 21 sampling events in which two-phase did not. The two-phase method detected WPV1 during 7 sampling events in which the BMFS did not. After cessation of SL2 use, it was more frequently detected in BMFS (4.2%) than two-phase (2.3%) samples (p=0.1) with the last detections of 2016 in June. Greater detection frequency in BMFS samples is likely due to the large effective volume assayed of the original sample (1590-mL BMFS vs. 150-mL two-phase).

Conclusions

This study demonstrated that the BMFS results in enhanced environmental PV detection in a polio endemic setting. Additionally, WPV1 detections in BMFS samples resulted in mass vaccination campaign responses by the WHO for timely interruption of virus transmission, even while the BMFS was being evaluated.

Prevalence of norovirus in produce sold at retail in the United Kingdom

Martin D'Agostino

CampdenBRI

One thousand one hundred and fifty two samples of fresh produce sold at retail in the UK were analysed for Norovirus. Of 568 samples of lettuce, 30 (5.3%) were Norovirus-positive. Most (24/30) lettuce samples which tested positive for Norovirus were grown in the UK and contained NoV GI. (19/24). Seven / 310 (2.3%) samples of fresh raspberries were Norovirus-positive. Most (6/7) of the positively-testing fresh raspberry samples were imported, but no predominance of a genogroup, or any seasonality, was observed. Ten / 270 (3.6%) samples of frozen raspberries were Norovirus-positive. The country of origin of the positively-testing frozen raspberry samples was not identified in most (7/10) instances. The collected data add to the currently limited body of prevalence information on Norovirus in fresh produce, and indicate the need for implementation of effective food safety management of foodborne viruses.

Towards routine analysis of infectious norovirus in bivalve shellfish

David Walker

Centre for Environment, Fisheries and Aquaculture Science

Introduction

Norovirus is a leading cause of gastrointestinal illness associated with the consumption of bivalve molluscan shellfish (BMS) worldwide. Current methodologies for quantifying norovirus in BMS rely on the use of quantitative reverse transcription PCR (RT-qPCR) based methods. However, these methods cannot discriminate between infectious and non-infectious noroviruses. Attempts to introduce guidelines and regulations to control the level of norovirus in BMS have been hindered in part by this lack of a suitable method for determining norovirus infectivity and thus its true risk to human health.

Objectives

In our role as the European Union Reference Laboratory for monitoring bacteriological and viral contamination of bivalve molluscs, Cefas have been investigating methodologies that could be adopted into routine analysis of BMS to quantify infectious norovirus.

Methods

In the first instance, we applied published methods designed to infer viral infectivity by exclusion of viral particles without intact capsids. Faecal samples containing norovirus were inactivated by UV radiation or heat alongside the F+RNA bacteriophage GA, which was used as a culturable surrogate virus. Additionally, analysis of approximately 600 BMS samples was carried out to determine the suitability of GA as an innate surrogate for norovirus in BMS. Using a combination of RT-qPCR and culture-based methods, the proportion of infectious GA was estimated and applied to norovirus as a means to estimate norovirus infectivity risk. The method previously used for quantification of infectious GA is laborious and time-consuming and so a new MPN-PCR based method was developed to further refine the use of GA as an innate surrogate in routine monitoring.

Results

Tests of virus samples treated to similar UV levels used in waste-water treatment plants, revealed that methods for inferring viral infectivity by capsid integrity analysis greatly underestimated the level of viral inactivation and were no better at quantifying infectious norovirus than RT-qPCR alone. Further experiments using GA to estimate norovirus infectivity in BMS suggested that BMS samples linked to norovirus outbreaks tended to have both high proportions of infectious GA and high overall levels of norovirus (as detected by RT-qPCR). Refinements of the method for quantitative detection of infectious GA in BMS reduced the time taken to obtain results from approximate 72 hours to approximately 36 hours.

Conclusions

The use of capsid integrity methods for inferring viral infectivity can lead to significant overestimation of infectious virus where inactivation is primarily linked to UV irradiation, and their use for routine monitoring for BMS is therefore not advised. On the other hand, while the use of culturable surrogate viruses may not completely eradicate errors in quantifying infectious norovirus, they may provide a tool for practical assessment of the probable level of risk associated with a sample and could therefore be adopted for use to reduce public health risk to consumers of BMS.

KEYNOTE SPEAKER:

Viral populations and quality control of irrigation waters

Marta Rusiñol, Xavi Fernandez-Cassi, Natalia Timoneda, Eloy Gonzales-Gustavson, Josep Francesc Abril, Gertjan Medema, Calvo M, Sandra Martínez-Puchol, Sylvia Bofill-Mas, Rosina Gironés

University of Barcelona, Spain

Objectives

The main aims are part of the METAWATER project: i. to evaluate the viruses present in irrigation water (tertiary effluents, river water and groundwater) and wastewater as a representative of the excreta of the population. ii. Evaluate the efficiency of WTPs and the reduction required for achieving acceptable risks for water reuse in irrigation of fresh vegetables.

Methods

During one-year period, 72 irrigation water samples were analyzed using SMF. Human viral pathogens and indicators and animal viruses used as MST tools were quantified by q(RT)PCR. To study the virome of irrigation water we developed a metagenomics protocol using Nextera XT and Illumina MiSeq 2x300bp.

Results and Conclusions

Both HAdV and JCPyV, human-specific fecal markers, were detected in all raw sewage samples. In general, NoVGGII concentrations were higher but the number of positive samples was lower than for HAdV. MCPyV was detected in both sewage and river water. HEV was detected in groundwater by qPCR and NGS, from an intensive pig farming area, where porcine specific indicators, PAdV, were also detected. The output of the NGS sequencing resulted in an average of 1,28 million reads describing the viral populations.
The results of the QMRA studies show that the disease burden of NoV GII and HAdV for the consumption of lettuce irrigated with tertiary effluent from two WWTPs was higher than the WHO recommendation of 10^{-6} DALYs suggesting that additional treatments are needed.

Inactivation of porcine viruses in the production process of spray dried porcine plasma

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Objectives

Spray-dried porcine plasma (SDPP) is commonly used as a protein ingredient in diets of farm animals and pets. Recently, it has been reported that infectious Porcine Epidemic Diarrhea Virus (PEDV) could be isolated from several feed ingredients, showing that PEDV can retain viability in extreme environments. To determine to what extent the infectivity of viruses in porcine plasma is reduced in the production process of SDPP before shipping, virus inactivation was studied on laboratory scale at the thermal and physical conditions used in spray drying.
The aim of this study was to determine of the thermal and physical conditions for inactivation of viruses, including adenovirus (AdV), porcine circovirus 2 (PVC2), porcine sapelovirus (PSV1) and PEDV, in the production process of the SDPP ingredient.

Methods

Citrate-treated porcine plasma of pH 7.5, 9.8 and 10.2 was spiked with PEDV, AdV, PVC2 and PSV1, and incubated at 3°C for up to 24 hours, and at 44°C or 48°C for maximum 10 min. Furthermore, virus-spiked concentrated plasma of pH 7.5 and 9.8 was spray dried in a laboratory scale apparatus at outlet temperatures of 80°C and 90°C. Aliquots of produced SDPP were stored for 0 to 10 weeks at 11°C and 20°C.

Results

Quantitative (RT)-PCR analysis detected no notable reduction in viral genomes in all heat-treated plasma and produced SDPP samples, and no infectious PSV1 and PCV2 could be re-isolated. Overnight incubation at pH 10.2 and 3°C reduced infectivity of PEDV in plasma to an undetectable level, whereas heating to 44°C at alkali pH was needed to reduce AdV infectivity effectively. Spray drying reduced AdV infectivity to an undetectable level and PEDV infectivity for 95%. After storage at 20°C for 2 weeks no infectious PEDV could be re-isolated from SDPP powders.

Conclusions

The thermal and physical conditions were determined to reduce infectivity of animal viruses in the production process of SDPP. These conditions differed markedly for AdV, PSV1 and PEDV. A considerable fraction (5%) of PEDV stayed infectious after spray drying. Applying a storage period of ≥2 weeks at 20°C further reduced infectivity of PEDV in SDPP to an undetectable level (log10-reduction factor of ≥4.0).

Determining viral genome functionality loss after various disinfection treatments using a novel transfection-based most probable number assay

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Ecole polytechnique fédérale de Lausanne

The primary mechanism of viral inactivation differs for disinfection processes (disruption of protein capsid, genome damage, etc.); accurately determining these mechanisms can aid the development of more robust water treatment systems. In this study, we developed a quantitative most probable number (MPN) transfection assay where viral RNA is inserted directly into host cells to directly measure the loss of virus genome functionality (i.e., ability to produce new progeny virus) when subjected to several disinfection methods (ozone, UV and free chlorine). These reductions were compared with reductions by qPCR and infection by cell culture.

Echovirus 11 (final concentration: 10^5 - 10^6 MPN/mL) was subjected to ozone, UV and free chlorine disinfection processes in phosphate buffered saline (pH 6.5 for ozone, pH 7.5 for UV and free chlorine). Samples were taken for each disinfection method at specified exposure levels. Virus infectivity was determined on BGMK cells. Extracted RNA and genome functionality was quantified using a novel Lipofectamine-based transfection MPN method and SYBR green RT-qPCR. Reductions were calculated by comparing to a non-treated control.

Reductions of replicable echovirus 11 RNA by ozone were observed for both transfection and qPCR based methods, however qPCR reductions extrapolated to the full genome were unrealistically high (max 55.90 $\log_{10}$) when compared to the corresponding reduction of infection MPN (max 3.99 $\log_{10}$) or transfection MPN (max 2.76 $\log_{10}$). These results were in contrast to those observed for UV treatment, where reductions were comparatively similar for the three methods. Experiments for free chlorine are ongoing.

Based on these results, transfection methods can be used to directly measure losses in viral genome functionality, and are more comparable to actual infection reductions than qPCR reductions for oxidative disinfection processes such as ozone.

Suitability of bacteriophages as surrogates for virus mitigation by electrocoagulation

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Objectives

Waterborne viruses are a ubiquitous cause of illness worldwide. Recently, electrocoagulation (EC) has shown promise in mitigating viruses in drinking water. However, a broad range of viruses has not been evaluated, and the influence of water quality on virus reduction by EC remains largely unexplored. The goal of this research was to determine the suitability of bacteriophages as surrogates for human viruses for testing the effectiveness of EC in a varying water matrix.

Methods

Laboratory-scale, iron electrocoagulation was tested for the reduction of bacteriophages and mammalian viruses from synthetic drinking water representing a range of water quality parameters: pH, natural organic matter (NOM), turbidity and chloride. EC was evaluated for the mitigation of three viruses: adenovirus 4 (ATCC VR-1572), echovirus 12 (ATCC VR-1563), and feline calicivirus (ATCC VR-782). In addition, four bacteriophages were used as virus surrogates: MS2 (ATCC 15597-B1), fr (ATCC 15767-B1), P22 (ATCC 97540) and ΦX174 (ATCC 13706-B1). Inactivation and total removal of both bacteriophages and mammalian viruses were quantified by cultural methods.

Results

The four viruses and three bacteriophages studied were mitigated to varying degrees by EC. While mammalian viruses were inactivated only at low pH, bacteriophages MS2 and fr were inactivated to a significant degree at all pH levels. Only ΦX174 was conservative in representing virus inactivation. Of the water quality parameters tested, pH had the strongest effect for all bacteriophages, while NOM impacted mammalian viruses to a greater degree than bacteriophages.

Conclusions

The sensitivity of common bacteriophage surrogates to inactivation by iron oxidation will likely overestimate virus mitigation. These results should inform not only electrocoagulation studies, but also research into other technologies using zero-valent and ferrous iron for virus mitigation.

Assessment of bacteriophage MS2 removal and contaminant reduction by biosand filter

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The objective of this study was to assess the role of nitrate and ammonia concentrations in the feed water on the removal of bacteriophage MS2 using the biosand filters (BSFs) and formation of the bacterial community in BSFs. Five bench-scale columns were constructed from PVC. Two BSFs were fed with high and low nitrate concentrations (50 mg/L and 4mg/L), while the other two BSFs were fed with high and low ammonia concentrations (35 mg/L and 4mg/L) for 48 weeks. The fifth column was fed with 0.5 mM of bicarbonate buffer and used as the control. The nitrate and ammonia concentrations reflected the water quality seasonality in Nepal. The experiment periods were divided the first period until 30 weeks and the second period after 30 weeks. The nitrate and ammonia concentrations in the feed water was switched from higher to lower concentration, and vice versa. The control filter showed the average MS2 removal of 4.09-$\log_{10}$ and 3.52-$\log_{10}$ at the first port and the outlet for 48 weeks, respectively. The BSF fed with low and high nitrate showed the average MS2 removal of 3.21-$\log_{10}$ and 3.52-$\log_{10}$ at the outlet and the MS2 removal showed better efficacy at the outlet than the first port. The filter fed with high ammonia showed stable MS2 removal and lowest with the average of 2.66-$\log_{10}$. A significant amount of ammonia was removed by the BSFs fed with 4 mg/L and 35 mg/L ammonia while the change in concentrations of nitrate in other two BSFs was not significant. The bacterial community was analyzed by QIIME2. These results suggest that the concentrations of nutrients can influence the biofilm growth inside BSFs and can have a significant impact on virus removal.

Identification of bacterial genes for producing norovirus-binding glycoconjugate

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Introduction

Human norovirus is the major cause of non-bacterial epidemic gastroenteritis. Norovirus infections occur via personal contact or by consuming food and water contaminated with human noroviruses. Patients having norovirus infection excretes virus particles with feces in high numbers. These excreta containing norovirus are collected and treated in municipal wastewater treatment plants. Studies have shown that, noroviruses are not completely eliminated from the treated wastewater. Wastewater effluent containing noroviruses discharged to natural waters or reused for other beneficial purposes can therefore act as a source of noroviruses. In fact, outbreaks due to consumption of oysters, strawberry and romaine lettuce contaminated or internalized with norovirus through environmental and irrigation water are reported frequently. This leads to the question that “how noroviruses survive persist and circulate in aquatic environments for longer time periods without being inactivated by environmental stresses?” However, this question has not been completely answered yet.

Recent studies have found that histo-blood group antigens (HBGA)-like substances possessing bacteria as a possible key player in determining the fate of human norovirus in aquatic environments. HBGAs are carbohydrates determining the ABO and Lewis blood types and can be found on mucosal epithelial cells and excreted in body fluids, including the saliva of secretor-positive (Se+) individuals. Human noroviruses recognize HBGAs for cell attachment and different norovirus strains have shown different HBGA-binding patterns. Similar to epithelial HBGA in human intestine, HBGA-like substances available in human enteric bacteria present in aquatic environments like wastewater act as specific receptors for human norovirus. Several studies have shown the effects of these specific interactions on the survival of norovirus in aquatic environments. Association with HBGA-positive bacteria has shown to protect human noroviruses from acute heat stress which is advantageous for norovirus while the same association contributed to the genotype-dependent removal of human norovirus by membrane filtration providing an advantage from public health point of view.

Therefore, analyzing the behavior of HBGA-positive bacteria in water environments may provide valuable information necessary to completely understand mechanisms of human norovirus persistence, circulation and survival. However, it is impossible to evaluate all the HBGA-positive bacteria on their contribution towards norovirus fate and circulation in aquatic environments due to the large number of bacterial strains present and other difficulties like viable but non-cultivable status of HBGA-positive bacteria. Identification of bacterial gene(s) responsible for HBGA-like substances production will provide an opportunity to utilize them as genetic markers to confirm the presence of HBGA-positive bacteria in aquatic environments. Since norovirus specifically interacts with HBGA-positive bacteria, evaluating the behavior of HBGA-producing gene may provide information on the behavior of human norovirus in the particular environment.

Objective

This study was performed to identify bacterial genes responsible for producing glycoconjugates containing HBGA-like substances.

Materials and methods

We used the Keio collection of *Escherichia coli* knockouts in our study. Keio Collection is a library of 3985 strains which are precisely defined, single-gene deletions of all nonessential genes in *E. coli* K-12

BW25113. Blood typing kit test showed that wild type *E. coli* K-12 BW25113 strain has a strong H activity. Based on the structure of H-antigen (Fucα1-2Galβ1-4GlcNAc-R), we selected genes that are involved in fucose (fuc), galactose (gal), and beta-glucoside (bgl) metabolisms from the Keio collection strains. Since HBGA-like substances are located in extracellular polymeric substances (EPS), genes in colanic acid (CA) cluster, which is a representative component of EPS, was also selected. H-like antigen production of selected strains was quantified by ELISA with anti-blood group H-antibodies. Effect of the reduced H-like substances on norovirus binding ability was evaluated by ELISA using GII.6 norovirus-like particles (NoVLPs).

Results

In total, 8 "*⊿fuc*" strains, 9 "*⊿gal*" strains and 7 "*⊿bgl*" strains were identified from the *E. coli* K-12 BW25113 genome and their gene functions were confirmed using Universal Protein Resource (UniProt) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. Considering CA, 22 mutant strains were selected. Out of the *⊿fuc*, *⊿gal* and *⊿bgl* strains, *⊿fucT*, *⊿galF*, *⊿galU*, *⊿bglB*, and *⊿bglG* displayed significantly lower ($p<0.05$) H-like antigen level than the wild type strain. Out of 22 CA related strains, 3 (*⊿wcaA*, *⊿wcaK*, *⊿asmA*) displayed significantly lower H-like antigen levels ($p<0.05$). However, blood typing kit was able to confirm the reduction of H-like antigen activity only in *⊿galU* strain. Binding assay performed using GII.6 NoVLPs showed only *⊿galU* strain has a significantly weak binding ability ($p<0.05$).

Conclusions

General enzyme UTP glucose-1-phosphate uridylyltransferase is encoded in *galU*. Even though *galU* may not be specific for H-like antigen production, it has a significant contribution to the bacteria-norovirus binding behavior. A further analysis on the contribution of *galU* in HBGA-like substances production in other enteric bacterial strains will provide valuable insights in understanding the influence of HBGA-like substances on the environmental behavior of human norovirus.

Challenges of infectious hepatitis E virus detection (in food products)

Willemijn Lodder, Saskia Rutjes

RIVM (National Institute for Public Health and the Environment)

Purpose/Objectives

Hepatitis E virus (HEV) RNA is detected in many diverse samples/matrices. Viral RNA has been detected in surface and irrigation water, in feces, liver and meat from pigs, deer and wild boar, and in human blood products. However, to be able to indicate whether exposure to these contaminated products may raise a public health risk, information on the viral infectivity is required. Therefore, a method for the detection of infectious HEV has been developed.

Materials/Methods

A549/D3, PLC/PRF/3 and HepG2/C3A cells have been grown in monolayers. The cells were inoculated with the supernatant of chronically infected A549 cells, as well as virus extracted from spiked livers (5 gram) and liver sausages (2 gram). In addition, the effect of an additive (FK506) that has been described to enhance the viral RNA concentration during cell culture was studied. Intracellular virus was detected using an immunofluorescence test (IFT). Viral RNA was detected by RT-qPCR. Amplification was estimated by comparing the amount of RNA detected at day 0 and after 14 days of incubation.

Results & Conclusions

We have shown the intracellular replication of HEV in cells grown *in vitro*. All three cell-lines were capable to replicate the virus, as was demonstrated by immunofluorescent staining of HEV particles in the cells. Both the inoculation of the virus supernatant as well as the virus extracted from spiked liver (sausage) resulted in positive IFT signals in A549/D3 cells, as well as a reduction of Ct values obtained by RT-qPCR after incubation, indicating an increase in HEV RNA. Adding FK506 seemed to enhance the virus replication.
The method will be optimized further to study HEV RNA positive animal food products, for instance liver and dried sausages such as farmer's sausage for the presence of infectious HEV. Both products have been identified as risk factors for hepatitis E.

Detection of foodborne enteric viruses in Irish oysters intended for human consumption

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Purpose/Objectives

Bivalve shellfish grown in coastal areas impacted by effluent from wastewater treatment plants can become contaminated with human pathogenic viruses. Such shellfish can represent a risk when consumed raw or inadequately cooked. A 12-month surveillance study was conducted to assess the occurrence of norovirus (NoV), sapovirus (SaV), hepatitis A (HAV), and hepatitis E (HEV) viruses in Irish oysters intended for human consumption i.e. oysters originated from EU category A areas or category B areas, following depuration.

Methods

Oyster samples (n = 150) collected bi-weekly (April 2017 - March 2018), from seven shellfish producing companies, were processed following ISO 15216:2017. NoV and HAV were quantified using the ISO 15216-1:2017 real time RT-qPCR, SaV as per Oka et al., (2006) and HEV as per Garson et al., (2012) & Jothikumar et al., (2005).

Results

NoV was detected 61.33% of samples. The concentration ranged from undetected to 1260 copies/g of NoV GI and undetected to 1411 copies/g of NoV GII. The prevalence of NoV GI (53%) was higher than NoV GII (36.67%). Seasonality was evident with 80.9% prevalence of NoV during the months of October to March compared to 36.3% during April to September. Overall, 70% of samples tested contained low levels of NoV (<LOQ).
Sapovirus prevalence (20%) exhibited a wide range between shellfish companies. No samples tested positive for HAV, while HEV testing is on-going.

Conclusions

Concentrations of norovirus (GI and GII) detected in shellfish were generally low (<LOQ), though prevalence was high during the winter period. The public health risk associated with these oysters remains unclear due to the uncertainty surrounding infectious dose of NoV from contaminated food and the lack of a routine cell culture system for NoV. . However, there were no reported outbreaks associated with these samples in Ireland or European RASFF alerts (2018). SaV displayed both a temporal and seasonal influence, while the absence of HAV supported the human epidemiology data within Ireland.

Molecular epidemiology of Hepatitis E Virus (HEV) in human and swine populations in Ireland

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Purpose/Objectives

In 2016, HEV, a significant zoonotic pathogen, became a notifiable disease in Ireland. The dominant autochthonous genotype of HEV in Europe is genotype 3, which has been associated with consumption of contaminated food, specifically pork products. As little is known about the epidemiology of HEV in Ireland, the Department of Agriculture, Food and the Marine funded the FoVIRA study to investigate HEV molecular epidemiology. We describe for the first time the molecular characterisation of HEV in human and swine samples in Ireland.

Materials/Methods

Serum samples, from symptomatic patients, received at the National Virus Reference Laboratory in 2016/2017 for HEV PCR were identified, prospectively. Additionally, fecal swabs were collected from swine at 4 farms in 2017. Sanger sequencing of a 493 nucleotide fragment of the ORF2 region of the HEV genome was performed on 35 samples (23 human, 12 porcine). HEV genotypes were analysed and compared by maximum-likelihood phylogenetic analysis.

Results

HEV G3 was the dominant strain (34/35) identified in both human and swine, with one travel-associated human case of HEV G1. Consistent with reports from the UK, 2 distinct clusters were observed in the human cases. A number of HEV G3 subtypes were identified; 3c (n=10), 3e (n=6), 3f (n=4) and 3untyped (n=2). Of note, all porcine samples were characterised as G3e (n=12).

Conclusion

This data demonstrates the dominant genotype of HEV in Ireland in 2016/2017 was HEV G3 with evidence to suggest that although a number of subtypes are in circulation in human cases, there may be less variation in the swine population. This preliminary study will be expanded upon to incorporate pork products at retail and an increased number of farms to better understand the risk of foodborne transmission of HEV in Ireland.

Sapovirus and Aichi virus associated to imported shellfish from developing countries

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Objectives

The risk of infection by foodborne diseases has increased due to the international trade of food products, including shellfish. Bivalve molluscs naturally concentrate pathogens due to their filter-feeding nature, becoming important vectors for viral gastroenteritis. In recent years, novel enteric viruses have emerged associated with the human consumption of these foods. The aim of the present study was the detection, quantification and genetic characterization of Sapovirus and Aichi virus from shellfish samples imported into Spain.

Methods

The study included 54 shellfish samples imported with commercial purposes, between September 2006 and January 2011, into Spain from Morocco, Peru, Vietnam and South Korea. In addition, some samples obtained from a street stall in Melilla (Africa). Species studied were clams (*Callista chione, Transanella pannosa*, and *Donax sp*.), oysters (*Crassostrea angulata*), scallops (*Pecten maximus*), cockles (*Cerastoderma edule*), and razor clams (*Solen marginatus* and *Ensis sp*.). Techniques employed were RT-qPCR for detection and quantification, and RT-nested PCR for further Sanger sequencing and genotyping.

Results

Sapovirus (SaV) were detected in 29 samples (53.7%): 12 from Morocco (22%), 11 from Peru (20%), 2 from Vietnam (4%), 1 from South Korea (2%) and 3 (6%) from Melilla; whereas Aichi virus were detected in 18 samples (33.3%): 12 from Morocco (22%), 3 from Peru (6%), 1 from Vietnam (2%), 1 from South Korea (2%) and 1 (2%) from Melilla. Of these samples, 10 were positive for the two studied viruses. The genotyped SaV positive samples corresponded with 10 sequences of GI.2 and 2 of GI.1, while genotyped Aichivirus positive samples comprised 5 genotype A and 1 genotype B sequences.

Conclusions

Due to the lack of routine viral analysis for bivalve molluscs, the global trade of these food products became an important route for international transmission of these pathogens. Therefore, they constitute key factors for the appearance of viral foodborne outbreaks with important implications in human health.

Human health risk associated with crAssphage gene marker in fresh untreated sewage- and tertiary effluent- contaminated recreational water

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Objective

Sewage-associated marker genes are used to assess sewage contamination in receiving waterways when the direct measurement of multiple pathogens is infeasible. Quantitative microbial risk assessment (QMRA) modeling can facilitate the interpretation of MST data in terms of the risk of gastrointestinal (GI) illness caused by exposure to pathogens.

Methods

In this study, a QMRA analysis was undertaken based on a newly developed crAssphage MST marker to determine critical concentrations in water samples that reflect a significant health risk when recreational water is contaminated with untreated sewage or treated effluent. The QMRA models were evaluated at the gastrointestinal illness risk benchmark probability of 0.036 (36/1,000) for the reference pathogens norovirus (NoV).

Results and conclusions

crAssphage markers at several dilutions exceeded the risk benchmark for the reference pathogen NoV. Concentrations of crAssphage on the order of 5.68×10^3 to 2.13×10^4 GC/100 of recreational water represented a significant health risk to swimmers from NoV, with both untreated sewage and treated effluent. Results of this study provide a valuable context for water quality managers to evaluate human health risks due to recreational exposure to waterbodies associated with contamination from fresh sewage. The approach described here may also be useful in the future for evaluating health risks from contamination with other animal sources as more data are made available.

Rotavirus uptake rate and decay rate for arugula (Eruca sativa) internalization determined for quantitative microbial risk assessment

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Water reuse for irrigation is becoming more common as water resources are strained by the increase of human population and food needs. Pathogens from contaminated irrigation water could be carried to crops, resulting in foodborne infection risks, especially fresh produce eaten raw. Viruses are the major cause of foodborne disease for leafy vegetables. It has been shown that viruses can internalize vegetable tissues when vegetables were grown with virus containing irrigation water. When this happens, sanitation of vegetable with a disinfectant such as chlorine cannot be effective as the internalized viruses are protected by the vegetable tissues from a disinfectant. Therefore, associated viral infection risks could be high. However, it remains unknown how much viral infection risks are associated with consumption of vegetable internalized by viruses. In this study, we aimed to obtain input parameters that are needed for risk assessment of consumption of vegetables internalized by viruses with experiments, such as virus uptake rate from feed water to vegetable tissues and virus decay rate after harvest. We conducted these experiments above using arugula (Eruca sativa) grown with hydroponics, with roof-harvested-rainwater, which has been gaining attention as an alternative irrigation water source. Rotavirus group A, which is the major cause of acute gastroenteritis for children under age of five worldwide, was chosen as our model virus. Especially, strain OSU (G5P[7]) was used.

In this presentation, we will provide laboratory scale-obtained input parameters for risk assessment and estimated risks associated with consumption of vegetables internalized by rotaviruses.

Acanthamoebidae and mimiviridae sequences detected in the feces of puerto rican pre-columbian cultures

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Purpose/Objectives

Free living amoebae (FLA) are one of the most prevalent protozoa detected in the environment. *Acanthamoeba spp.* serve as a reservoir for potential human pathogens known as amoeba resistant bacterias (ARB) along with a putative pneumonia agent *Mimiviridae*. The protozoan/giant-virus interaction was most likely present over 1,500 years ago since we identified *Acanthamibidae* and *Mimiviridae* sequences in pre-Columbian coprolites.

Materials/Methods

Ancient DNA from human feces of two pre-Columbian indigenous cultures was isolated and sequenced using shotgun metagenomic sequencing. The Huecoid and Saladoid datasets were assessed and identified *Acanthamibidae* and *Mimiviridae* sequences using BLAST alignment (cut-off value set at E value <-15).

Results

A total of five *Acanthamibidae* sequences with homology to *Acanthoamoeba castellanii* were identified in the Huecoids (n=2) and Saladoids (n=3) datasets. A total of 14 *Mimiviridae* homologous sequences were identified in the Huecoids (n=4) and Saladoids (n=10) datasets.

Conclusions

Considering the distribution and diverse ecological niches of *Acanthamibidae* and regarding that *Acanthamoeba* is not a component of modern humans fecal microbiota, detection of *Acanthamibidae* in pre-Columbian coprolites was due to ingestion of contaminated food source or untreated water source. Seeing as how bromeliads harbor both FLA (including *Acanthamoeba spp.*) and other microorganisms phagocytized by the FLA, we are considering bromeliads as a possible alternative source of drinking water and food source for the indigenous cultures. This is the first report of *Acanthamibidae* and their associated giant viruses found in ancient feces.

Detection of noroviruses and hepatitis E virus in pork meat product chain

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Transmission of viruses via food and the environment is now a well-recognised however neglected problem. In the EU, viruses were identified as the second most common causative agent of the foodborne outbreaks and an increased number of such outbreaks has been described. Although several groups of viruses may infect persons after ingestion of contaminated food, noroviruses (NoV), hepatitis A virus (HAV) and hepatitis E virus (HEV) are currently recognised as the most important foodborne pathogens.

Since the viral contamination of food can occur during all stages of any supply chain, present study was focused on safety of the pork meat products. Environment of the pork meat production chain (meat processing plant and four charcuteries), food handlers and pork meat products (deli meat, products of home slaughter and minced meat) were tested for presence of NoV, HAV and HEV by RT-qPCR.

At the production level, swabs of hands and gloves of food handlers (64 samples), equipment (32) and rest room (8) were NoV negative. NoV were detected only in swab from toilets (1/32). Possible failure in deployment of GMP a GHP was found out in the charcuteries; NoV was detected on the hands of sellers (1/8), equipment (4/80), toilets (1/32) and in the deli meat (6/135), products of home slaughter (5/145) and minced meat (3/100).

HEV was not found in any tested swab or deli meat. Presence of HEV in product of home slaughter (1/145) and minced meat (1/100) suggest primary contamination or cross contamination during the processing. HAV was not detected in any analysed sample.

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Quantitative detection of human adenovirus in wastewater treatment plant influencing the Mississippi river in Louisiana

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Introduction

The Mighty Mississippi River is an important natural resource in the United States since it is a source for drinking water in many cities, as well as for recreational, agricultural, and industrial purposes. However, there is a well-documented history of contamination along its path into the Gulf of Mexico. Untreated wastewater is one of the major concerns in relation to water bodies used for recreational activities and seafood harvesting in coastal Louisiana. qPCR approach will be utilized to assess human adenovirus (HAdVs) removal in wastewater treatment plant which releases treated wastewater to the Lower Mississippi River in Louisiana.

Objectives

To assess HAdVs removal in a conventional wastewater treatment plant in Southern Louisiana

Methods and Results

Electronegative membrane-vortex method was used to concentrate the wastewater samples. Two hundred microliters of the virus-concentrated sample were subjected to viral DNA extraction using a Zymo Extraction kit. HAdVs were then detected only by TaqMan quantitative PCR and HAdVs were detected in 100% of raw influent. The average HAdVs loads were significantly reduced along the treatment trains but HAdVs were still present in the final effluent.
This presentation will cover the findings that indicate adenoviruses are not completely removed by this treatment plant and suggests a potential public health risk.

Analysis of fecal source of contamination using viral indicators in Santa Lucia and Uruguay rivers

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Background

Fecal contamination of watersheds can cause serious consequences for the environment and public health.

Purpose

The aim of this work is to determine precisely the origin, whether human, bovine or porcine, of the different fecal contamination sources in the waters of the Santa Lucía and Uruguay River watersheds, by the use of host-specific viral molecular markers.

Methods

Monthly collections of surface water samples during one year was performed in six sites in Santa Lucía River and four sites in Uruguay River (120 samples) between June 2015 and May 2016. Viral concentration was performed by using an absorption-elution method with a negatively charged membrane and nucleic acids were extracted with the *QIAmp Cador Pathogen kit*. cDNA synthesis was carried out with random primers for Group A Rotavirus (RVA). Detection and quantification of human (HAdV) and porcine (PAdV) adenovirus, human (HPyV) and bovine (BoPyV) poliomavirus and RVA was carried out by quantitative PCR.

Results

RVA was the most frequent virus identified (41% - 49/120) followed by HAdV (18% -21/120), BoPyV (11% - 13/120) and HPyV (3% -3/120), with no detection of PAdV at no sampling point. The mean concentration of RVA was $1.5x10^5$ genomic copies/L (gc/L), for HAdV was $1.5x10^4$ gc/L, for BoPyV was $1.1x10^4$ gc/L and for HPyV was $1.8x10^2$ gc/L.

Conclusions

This is the first study performed in Uruguay in order to get insight into the presence and the distribution of these host specific viral markers of fecal contamination. These results suggest that fecal contamination has a negative impact in the quality of the waters of these rivers, showing deficiencies in the procedure of sewage discharge from regional cities and in the breading of cattle.

Bag-mediated filtration system v2: Modifications and improvements

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Purpose/Objectives

The bag-mediated filtration system (BMFS) was designed for poliovirus environmental surveillance. Based on user and collaborator feedback received during the field validation of the first version of the BMFS (BMFS v1), the system underwent refinement to improve poliovirus recovery, sampling efficiency, biocontainment, and QA/QC mechanisms leading to the BMFS v2.

Materials/Methods

Modifications to the BMFS sample collection bag include eliminating the metal collar (needed for assembly) by incorporating the pre-screen into the bag, and making the bag transparent. The filter housing modifications include changing the port locations and increasing the void space to 150mL. The new elution device is now collapsible and accommodates two filter housings. The BMFS laboratory protocols have also been modified. The elution protocol now utilizes a double elution (15 minutes each) while the secondary concentration step involves skim milk flocculation (vs PEG/NaCl precipitation). Disposable items have been incorporated elsewhere in BMFS v2. The effect of these modifications on poliovirus recovery was determined by seeded laboratory experiments.

Results

Different elements of the BMFS v1 and v2 kits and protocols were compared to evaluate their effect on poliovirus recovery. The sample collection bag reduced potential user error. The redesigned filter housing simplified assembly, improved fluid flow, reduced filtration time, and eliminated leaking. The new elution device modifications improved ease of use and reduced processing time. Skim milk flocculation eliminated the need for overnight refrigerated shaking, resulting in faster processing and simpler equipment needs. BMFS v2 refinements resulted in an improved lower-cost system with increased poliovirus recovery ($p<0.05$) and all modifications addressed field and laboratory staff feedback.

Conclusions

The improved poliovirus recoveries, combined with the additional benefits in ease of use, manufacturing, and cost that were obtained with BMFS v2 have prepared the system for verification by international collaborators and commercialization.

Metagenomics for the detection and characterization of HEV in water, food and clinical samples

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Objectives

Mass sequencing protocols were optimized and applied to the identification and characterization of the viruses excreted in urban sewage that could contaminate irrigation water and fresh vegetables being an etiological agent of acute hepatitis.

Methods

Sequencing protocols using paired end 300-base runs on the Illumina MiSeq platform with improved sample pre-treatments and bioinformatics pipelines were developed and applied to viral particles concentrated from sewage and groundwater samples as well as to parsley plants which had been irrigated with river water.

Results and Conclusions

Forty-one viral families, including pathogenic viral species from 7 families were identified in urban sewage collected in the area of Barcelona: HEV-3, was identified in 2/5 sewage samples analysed.

Interestingly, parsley plants irrigated with urban river water from the same area presented several sequences related to HEV-3 ranging from 154 to 548 bp long which matched up with variable identities at nucleotide levels ranging from 86 to 98% and clustered with HEV-3f.

In addition, in a study analyzing irrigation water in Europe (METAWATER), groundwater samples from an area with porcine farms and porcine adenoviruses in 4/12 samples analyzed, presented HEV in 1/12 of the samples analyzed.

The metagenomics protocol developed was also used to study pools of serum samples from 10 acute hepatitis patients infected with HEV in the same area. A total of 27 contigs were matched to sequences of the *Hepeviridae* family, 76.1% (5,508 of 7,238 bp) of the HEV whole genome was sequenced with an average pairwise identity of 85.5% against the genotype HEV-3f and other closely related genotypes. In addition, 3 contigs from an immunossupressed patient's serums pool were also aligned with genotype 3a.

Viral metagenomics is a promising tool applicable to environmental surveillance, food-safety, outbreak investigation and clinical diagnostics of hepatitis patients.

Virwatest: a method for detection of viruses in water samples in the point-of-use

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Objectives

Viruses found in aquatic reservoirs are associated with health risks and responsible for human infections. The commonly used standards for the quality control on faecal contamination proposed and for monitoring effectiveness of disinfection measures are *E. coli* and enterococci, used as faecal indicator bacteria (FIB). But, there is little reason to believe that FIB can predict the presence of viruses which are more resistant to many inactivation processes.

Until recently, viral monitoring required complex logistics, a specialised team and shipment to a reference laboratory for analysis representing a low sensibility of methods due to lack of adequate experimental, storage and shipping conditions. An affordable and simple procedure for concentrating and detecting viruses in water samples and in the point of use is needed.

Methods

The VirWaTest method comprises three steps: concentration of viral particles present in water samples, nucleic acid extraction and detection by PCR-based methods. These steps have been adapted from methods currently in use in laboratories for being performed in the point of use by reducing the required equipment and the dependence on power supply and freezers. Preservative solutions have been added to the procedure to guarantee stability of nucleic acids for shipment to reference laboratories if needed.

Results and Conclusions

The developed method has proven to be equivalent to others commonly used for virus detection in water samples. When evaluated by Oxfam Intermon WaSH teams in Banghi (RCA) and Pedernales (Ecuador) has been useful for detection of viruses.

VirWaTest may be part of an early warning system for organizations involved in water sanitation and would make more affordable to implement the analysis of viral pathogens in water and the improvement of water safety management to reduce incidence of viral diseases in humanitarian crisis contexts.

The utility of flow cytometry in water reuse applications

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Purpose/Objectives.

One primary challenge of direct potable reuse (DPR) is the effective removal of pathogens present in wastewater. Human norovirus (HuNoV) is of principal concern in DPR because of its ubiquitous presence in wastewater and large disease burden. However, little is known regarding HuNoV infectivity through DPR treatment, because there is no widely accessible in vitro assay to measure HuNoV infectivity. Other methods that estimate HuNoV inactivation, including HuNoV genome concentrations and indicators/surrogates, have obvious limitations. More accurate inactivation data for HuNoV are necessary to ascertain if appropriate virus removal is achieved through DPR. Here, we assess HuNoV inactivation during UV treatment by targeting a large portion of the HuNoV genome and use a novel HuNoV culture system to quantify reductions of infective HuNoV through disinfection.

Methods.

UV-treated samples containing HuNoV are amplified using eight amplicons covering over half the HuNoV genome. The proportion of intact amplicons is extrapolated to the entire genome to obtain low-pressure UV inactivation curves for HuNoV. Disinfection kinetics are also determined by intestinal organoid cell-culture following infection with HuNoV taken from inactivation experiments.

Results.

Using genome extrapolation methods, HuNoV is significantly more susceptible to UV inactivation than MS2 bacteriophage and adenovirus. At the UV dose of 186 mJ/cm2, the minimum UV dose for 4-log inactivation of viruses in drinking water, over 20-log inactivation of HuNoV is observed.

Conclusions.

HuNoV is inactivated to a much greater extent than the viruses driving UV design in drinking water and reuse (i.e., adenovirus and MS2). UV treatments protecting against these viruses will provide even greater HuNoV log-reductions. HuNoV infectivity through other disinfection processes is being evaluated using the organoid system. These results can be applied to evaluate the treatment performance and risks associated with HuNoV in DPR treatment schemes.

Synthetic-long read next generation sequencing techniques for poliovirus identification in environmental samples

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Purpose/Objectives

Poliovirus (PV) environmental surveillance plays an important role in ensuring the completeness of the global eradication program. The bag-mediated filtration system (BMFS) was developed to enhance surveillance sensitivity. This study compared BMFS environmental surveillance results with the method recommended by the World Health Organization (WHO; two-phase separation). This study provided a unique opportunity to monitor the disappearance of Sabin-like PV type 2 (SL2) from the environment after global cessation of SL2 use in routine or supplementary immunization activities in April 2016.

Materials/Methods

From February 2016-present, BMFS (5.8±0.1-L) and two-phase (500-mL) samples were collected concurrently from 15 sites in 12 Pakistani districts (n=295), and analyzed for Sabin-like PV (SL), wild PV (WPV), and vaccine-derived PV (VDPV) types 1, 2, and 3 by the WHO algorithm.

Results

PV was detected in a majority of BMFS (83.7%) and two-phase samples (64.1%). Compared to two-phase samples, BMFS resulted in more frequent detection of SL1 (p<0.0001), SL2 (p=0.035), SL3 (p<0.0001), and WPV1 (p=0.0081). The BMFS detected VDPV2 during one and WPV1 during 21 sampling events in which two-phase did not. The two-phase method detected WPV1 during 7 sampling events in which the BMFS did not. After cessation of SL2 use, it was more frequently detected in BMFS (4.2%) than two-phase (2.3%) samples (p=0.1) with the last detections of 2016 in June. Greater detection frequency in BMFS samples is likely due to the large effective volume assayed of the original sample (1590-mL BMFS vs. 150-mL two-phase).

Conclusions

This study demonstrated that the BMFS results in enhanced environmental PV detection in a polio endemic setting. Additionally, WPV1 detections in BMFS samples resulted in mass vaccination campaign responses by the WHO for timely interruption of virus transmission, even while the BMFS was being evaluated.

Developing a method to specifically and simultaneously measure the concentration of infective enterovirus serotypes in environmental samples

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Objectives

The objective of this study is to selectively and simultaneously measure the infective concentration of sixteen enterovirus serotypes in environmental samples. This method will allow to monitor the dynamics of the enterovirus population distribution. The serotypes considered are representative of those predominantly occurring in European sewage samples.

Methods

Integrated Cell Culture coupled with quantitative polymerase chain reaction (ICC-qPCR) is used. In ICC-qPCR, the sample is incubated for a few hours on cell lines, allowing the infective viruses to replicate. After incubation, the propagation is stopped, the RNA is extracted and the concentration of replicated genomes is measured by qPCR. This method is specific to infective viruses, as the viruses replicate prior to detection by qPCR. The use of qPCR allows to selectively detect some viruses. Rhabdomyosarcoma (RD) and Buffalo Green Monkey Kidney (BGMK) cell lines were selected to propagate all the serotypes of interest.

Results

Serotype-specific primer pairs were designed based on the consensus of twenty published sequences per serotype. No more than two degenerate nucleotides were needed to ensure selectivity while retaining serotype specificity. The efficiency and specificity or each primer pair were determined, and standard curves for each serotype were created to link the qPCR signal of replicated genome to the infective virus concentration in the environmental sample. The method was tested on solutions of known virus composition, and on wastewater samples obtained from the Lausanne treatment plant.

Conclusions

We developed a virus detection method that yields information about the concentration and the distribution of infective enterovirus serotypes in environmental samples. Ultimately, this method will enable us to monitor how the enterovirus population shifts due to sewage treatment or environmental exposure.

Process of acquisition of UKAS accreditation for detection of norovirus GI&GII and hepatitis A in berry fruit and leafy greens

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Accredited methods serve the purpose of objectively proving to the customer competency and integrity of the laboratory performing the analysis. Virus testing, especially Norovirus/Hepatitis A testing in food produce is still not widely available. Only a minor percentage of laboratories offering the service have an internationally accepted accreditation. One of the global accreditation giants, UKAS lists 3 laboratories accredited in detection and quantification of Norovirus in bivalve molluscs and only one capable of performing the same analysis in fresh fruit and salad vegetables. Why is this so? Virus testing is quite different both to traditional microbiology and chemistry and requires specific equipment and technically skilled staff. Price of the analysis and lack of regulation means that no producer is obliged to perform the test which respectively discourages laboratories from setting up the method, let alone performing accreditation. In preparation for accreditation a designated Containment level 2 laboratory is required, followed by a suit of management level documents regulating workflow and
safety of both products and staff, especially when it comes to extraction of Hepatitis A virus. Validation trials are essential and as such take up considerable amounts of time and resources. Lastly analytical parameters such as Limit of Detection and/or Quantification, repeatability, accuracy and so on need to be established, which are much harder to do in practice than the corresponding ISO standard would suggest. Accreditation of Virus Detection/Quantification methods for food and food surfaces is now essential as it will further increase the trust of producers and retailers in analytical services offered, as well as increase consumer confidence.

Adaptation of a cationic bead norovirus extraction protocol to berry type matrices

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Canadian Food Inspection Agency

Introduction

Food viruses of clinical importance are among the most important foodborne pathogens recognized worldwide. The extraction and detection of foodborne viruses from berries are challenging due to the matrix high polyphenol content. These molecules are known to be strong RT-qPCR inhibitors. These molecules also appear to form complexes with pectin, a polysaccharide present in the fruit cell wall, and to reduce the efficiency of the extraction methodologies.

Objectives

We present parameters that were taken into account in the optimization of a silica-based method for the extraction of noroviruses. We also present the overall results of a recent recall involving contaminated raspberries, obtained using this methodology.

Methods

Human norovirus GII and murine norovirus were spiked on frozen blueberries, raspberries and strawberries. Recovery yields of human norovirus GII using a silica bead-based method and a modified ISO/TS 15216 protocol were compared. Parameters associated to the pectinase incubation step (time, concentration, buffer, temperature, pH) were modified.

Results

Pectinase incubation had a negative impact on recovery yield using the silica bead-based method. The recovery yield of the modified ISO/TS 15216 (4%) was clearly superior to the silica-based method (0.3%) with blueberries. The recovery yields from raspberries and strawberries were similar although inhibitors were occasionally present with the modified ISO/TS 15216 method. The recovery yields of norovirus GII from raspberries were 2.1±0.8% and 1.5±0.8% for the silica bead-based and the modified ISO/TS 15216 methods, respectively. The recovery yields of norovirus GII from strawberries were 2.4±0.8 and 1.8±0.8 for the silica bead-based and the modified ISO/TS 15216 methods, respectively. Twenty-five subsamples from 6 raspberry lots were tested positive by RT-qPCR using the silica bead-based method.

Conclusion

The capacity of surveillance agencies to detect foodborne viruses in berries will keep improving through continuous progress in the extraction methodologies and better knowledge of the matrix-virus interaction.

Human Intestinal Enteroids as a Model for Enteric Virus Infection

Samantha Wales, Zhihui Yang, Diana Ngo, Michael Kulka

Human Intestinal Enteroids as a Model for Enteric Virus Infection

Introduction

Human noroviruses (HuNoV), as well as other human enteric viruses, have been recalcitrant to growth in culture, prohibiting in-depth research into their replication toward development of virus infectivity-based detection assays. The development of a reliable cell culture model for human noroviruses viruses is vital, therefore, for confirming the presence of infectious virus in a potentially contaminated food source, as well as achieving production and consistent availability of HuNoV stocks for use in research and development of mitigation, food extraction, and detection methodologies.

Objectives

The objective of this study is to determine the potential and limitations of the human intestinal enteroids system for the growth of human norovirus strains and other selected enteric viruses.

Methods

We adopted the methods developed by Dr. Mary Estes' lab at Baylor College of Medicine using human intestinal enteroids seeded in a monolayer for infection with human noroviruses, astrovirus, and sapovirus.

Results

The GII.4/Sydney strain provided by Dr. Estes' lab demonstrated robust, consistent replication in the enteroids. At least ten other strains were also tested, but of these, only two exhibited signs of replication. Human astrovirus, but not sapovirus, was also able to replicate in this system.

Conclusion

The development of the human intestinal enteroids system for enteric virus infection has greatly progressed the field for norovirus replication, however it is limited in that few norovirus strains are able to grow. More work needs to be done to determine what factors are limiting the growth of other strains.

New insights for optimizing molecular detection of infectious viruses

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Introduction & Objectives

The use of molecular techniques for virus detection has become very common over this last decade. Nevertheless, virus molecular detection presents limitations. In environmental context, viruses are subject to various pressures impacting their infectivity and their persistence. In that respect, the detection of viral nucleic acids does not consistently imply infectious risk. Although several studies have reported positive viral analyses of food and drinking water, these studies did not focus on discrimination between infectious and non-infectious viral particles. Several studies showed that pre-treatment with monoazids allowed to assess the integrity of virus particles. Different molecules (EMA, PMA or PMAxx) were used in non standardized protocols. We previously showed that monoazids are able to block the amplification of double stranded DNA, but also double stranded RNA and even single stranded RNA genomes. The mechanism by which these dyes interact with nucleic acids is not clear and we suggested that the dyes could bind preferentially to double stranded structures, particularly in the case of single stranded RNA genome. The knowledge of the mechanism of interaction between monoazid and nucleic acids is essential for improving integrity assay.

Methods & Results

In this study, we compared monoazid effect on various single stranded nucleic acids (DNA or RNA) in denaturing and non denaturing conditions. ssDNA were designed in order to generate *in silico* stable secondary structures including hairpins and internal loops. We showed that the PCR inhibition effect of EMA treatment was dye-concentration dependent but also strongly linked to secondary structures of nucleic acids (ssDNA or ssRNA). On the contrary, in the case of treatment based on PMA or PMAxx, the PCR inhibition effect was independent of dye concentration in the range tested and did not require stable secondary structures suggesting that PMA and its derivatives could differently interact (or with a stronger affinity) with nucleic acids than EMA.

Conclusions

RNA structure of a single stranded whole genome is very difficult to modelize due to their size and possibilities of tertiary structures resulting from 2'-hydroxyl group of ribose. In addition, secondary structure predictions resulting from base-pairing greatly depends on the algorithm used. However virus infectivity assay based on molecular detection should preferentially target highly structured region of the viral genome containing IRES or extended hairpins.
Finally, a well designed pretreatment with monoazid is a useful tool in order to evaluate the integrity of viral particles and finally to better appreciate the risk for public health

Assessment of pathogens in treated wastewater intended for crop irrigation

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Objectives

The major objective of this study was to apply the latest methods for the assessment of pathogenic viruses, protozoa and bacteria in treated wastewater from different water reclamation facilities that provide this resource for crop irrigation

Methods

Samples of treated wastewater were collected multiple times from six different wastewater treatment facilities in Arizona. Treatment at four of the plants involved advanced Bardenpho processes followed by chlorination and de-chlorination. Two of the other facilities used ultraviolet light as the primary disinfection process. Samples were tested for human enteric viruses, viral indicators (pepper mild mottle virus and crAssphage) *Cryptosporidium*, *Giardia*, *Cyclospora*, *Salmonella enterica* and *Listeria monocytogenes*. Enteric viruses were assessed by an integrated cell culture real time polymerase chain reaction (ICC-qPCR) assay and protozoan parasites by USEPA Method 1623 with modifications for the analysis of *Cyclospora*. S. *enterica* and *L. monocytogenes* were recovered by filtration through Moore Swab followed by non-selective/selective enrichments and plating on selective media. Presumptive positives were confirmed using PCR/qPCR.

Results

None of the samples tested were positive for *S. enterica* or *L. monocytogenes. A*denovirus, enterovirus, and reovirus were detected in a facility after chlorination (sample size 40 to 100 liters) but not in the effluent, while viral indicators were detected by qPCR in most facilities. No protozoa were detected in 20 – 60 liters. The results of this study indicate that in most cases pathogen levels were below detection meeting current standards in Arizona for irrigation of food crops.

Conclusions

The absence of pathogens in recycled waters indicate the overall safety of the resources for crop irrigation and the suitability of the wastewater treatment processes to fulfill these goals.

Evaluating the performance of conventional and new viral indicator assays for human pathogenic enteric viruses in food and water matrices

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Standard protocols for the detection of human pathogenic enteric viruses in food and water involve the application of laborious steps to recover low virus titres usually present in complex environmental matrices. The use of indicator viruses (i.e., phages) for assessing the potential for contamination and exposure is considered a valuable alternative that encompasses multiple advantages and disadvantages. For instance, the ease and relative cost of phage detection is a major advantage over human pathogenic viruses. In addition, the analysis of F-specific RNA bacteriophages (FRNAPH) with structural similarity to many human enteric viruses can be applied to distinguish human from animal fecal contamination with various degrees of certainty. crAssphage, a novel bacteriophage, discovered by metagenomic data mining and reported to be abundant in and closely associated with human fecal waste, has shown potential for water quality management applications. Nevertheless, more studies are needed to evaluate its performance as a viral indicator. The plant virus pepper mild mottle virus (PMMoV) has been detected in high concentrations in wastewater and our group has demonstrated its potential as a conservative tracer of virus transport through aquifer material. So far, no single viral indicator is suitable to determine the presence of multiple enteric viruses. This study evaluated the performance of FRNAPH, crAssphage, and PMMoV qPCR assays in determining the efficiency of virus reduction through wastewater treatment processes and the presence of enteric viruses or fecal contamination in outbreak scenarios. Water concentrates obtained by NanoCeram filtration and ultrafiltration were analyzed for human noroviruses genogroups I and II, adenoviruses, FRNAPH-I and II, crAssphage, and PMMoV. Food (puree and scallops) and water (well water) matrices implicated in food and water borne outbreaks were investigated for norovirus, hepatitis A virus, adenoviruses, and the three viral indicators. Nonporous surfaces potentially implicated in a rotavirus outbreak were also analyzed for rotavirus, norovirus, adenovirus, and viral indicators. Our results reveal variations in performance for each viral indicator and the need to evaluate multiple viral indicators including the pathogens themselves under specific scenarios to obtain meaningful information. The PMMoV, crAssphage and FRNAPH assays could be suitable to evaluate virus reduction during wastewater treatment while the correlation observed between the PMMoV and crAssphage ($r^2 = 0.70$, $p<0.05$) assays could be used to predict virus transport in managed aquifer recharge operations. The FRNAPH-I and II assays are suitable to evaluate human fecal contamination, however the crAssphage assay was more efficient than the FRNAPH assay for tracing this source of pollution due to its relatively high sensitivity. In waterborne outbreak scenarios, the virus genomes of pathogens and indicators are relatively similar demonstrating adequate performance of the qPCR assays. In foodborne outbreak scenarios, the qPCR assays for viral indicators are suitable, however due to the complexity of implicated matrices the assessment of the viral pathogen and multiple viral indicators may be required for better interpretation of test results.

Hepatitis E Virus in the Aquatic Environment

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Hepatitis E virus (HEV) is an emerging pathogen affecting the liver. It causes acute hepatitis, which can be fatal in approximately 1% of the infected population but causes higher mortality rates in the immunocompromised and pregnant women (1). HEV was previously thought to cause UK infections in people who had travelled abroad to endemic areas, but it has been identified that autochthonous cases occur; specifically, with genotype III (GIII) HEV. Recently, a study of UK pigs identified an anti-HEV antibody seroprevalence rate of approximately 92.8%, suggesting that undercooked pork may be a significant route of transmission of GIII HEV to humans (2). Other evidence has also emerged which suggests that shellfish could also be a route of HEV transmission; with possible contamination routes including human sewage or farm and slaughterhouse waste run-off into water courses. In Scotland, a small number of samples of mussels harvested from five separate sites showed a contamination rate of 85.4% (3). A separate study also showed that 2.9% of a larger number of shellfish samples purchased in Scottish supermarkets contained HEV (4). However, a large study which assesses the presence of Hepatitis E virus in oysters at point of harvest across the UK has not yet been performed; this will be addressed within this PhD. In addition to quantification, any HEV-positive shellfish or water samples will be sequenced and subjected to phylogenetic analysis to determine whether the identified strains are genetically similar to those found in UK pigs or human sewage. The locations at which positive samples were collected will also be subjected to GIS analysis to determine the proximity to pig farms, slaughterhouses and human combined sewage overflows, and assess the contribution of these risk factors to aquatic HEV contamination.

Hepatitis E in Irish pork production

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Purpose/Objectives

Hepatitis E Virus (HEV) is a zoonotic pathogen associated with foodborne disease. Consumption of raw and undercooked pork products are major risk factors for transmission with swine and wild boar the main reservoirs. On farm studies of HEV infection dynamics in pigs are important to understand and manage public health risks. In this investigation the infection kinetics of HEV on Irish pig farms along with the prevalence of HEV in pig liver are described for the first time.

Materials/Methods

Serum and faecal samples were collected from commercial farms representing a cross section of the national herd at regular time points over a six month period. HEV IgG serology and molecular detection of HEV RNA was performed using PrioCHECK HEV Ab porcine ELISA and an in-house HEV RT-PCR assay. A point prevalence study was undertaken to determine the presence of HEV RNA in porcine livers at slaughter.

Results

In total 228 serum (IgG and RNA) samples and 182 faecal (RNA only) samples were tested. Although viral infection dynamics varied between herds, 93% (n=41) of animals tested at 20 weeks were HEV IgG seropositive and HEV RNA was not detected in any animal at this time point. Overall detection of HEV RNA was low (6%) with higher rates in faecal samples (11%) compared to serum samples (3%). Peak viral RNA detection was observed at 12 weeks of age. HEV RNA was not detected in any of the liver samples at time of slaughter.

Conclusions

This study confirms HEV is circulating in Irish pig herds. Infection kinetics suggest viral clearance occurs prior to slaughter. To date no evidence of HEV RNA in abbatoir samples has been detected. Further work is ongoing to assess the risk of HEV in pork products at point of retail and to investigate the molecular epidemiology of HEV isolated from pigs and humans in Ireland.

Estimating the Effect of a Quaternary Ammonium Hand Sanitizer on Norovirus Infection Risk

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Purpose/Objectives

The purpose of this study was to use a norovirus exposure model to estimate efficacies of a quaternary ammonium-based hand sanitizer in reducing norovirus infection risk and to identify conditions for which largest efficacies were estimated.

Materials/Methods

A discrete-event model accounting for hand-to-surface, -eyes, -nose, and -mouth contacts was used to estimate the effect of a single application of quaternary ammonium hand sanitizer on norovirus infection risk for 8 hours of simulated activity. An exponential curve was fit to mean $\log_{10}$ human norovirus (HuNoV; GII.4 Sydney) reductions measured on hands at 30s, 60s, 4 hrs, and 8 hrs after quaternary ammonium hand sanitizer application. This curve was utilized to simulate expected viral reductions on hands after hand sanitizer application. Simulated norovirus surface concentrations, ranging from 0 to 53,725 MPN/100cm^2, originated from surface samples from a norovirus houseboat outbreak. Per simulation, baseline and intervention cumulative doses and infection risks were estimated and used to calculate a percent change to evaluate intervention efficacy.

Results

Hand sanitizer application within the first 10 simulated minutes resulted in at least a 90% infection risk reduction. The greatest infection risk reduction (99.99999%) was estimated when hand sanitizer was applied at ~11.8 minutes, and baseline and intervention infection risks were 0.72 and 6.3 x 10^{-8}, respectively. The smallest infection risk reductions occurred in simulations in which hand sanitizer wasn't applied until ~5.9 hours or later and hand-to-mouth contacts happened to not occur after hand sanitizer application. As hand sanitizer application occurred later in simulations, cumulative dose and infection risk reductions decreased as residual reductions on hands occurred for smaller durations.

Conclusions

Optimally timed hand hygiene interventions with large initial $\log_{10}$ reductions and long residual product time may yield promising infection risk reductions.

Reduction of Aichi virus by blueberry proanthocyanidins in model foods and under simulated gastric conditions

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Introduction

Aichi virus is an emerging enteric virus with a single-stranded, positive-sense RNA genome enclosed in a non-enveloped capsid that causes human gastroenteritis outbreaks worldwide. Natural control measures to prevent their spread and alleviate disease symptoms are needed, as commercial vaccines are not currently available. Blueberries and blueberry polyphenols are increasingly consumed for their health benefits, high antioxidant value and antimicrobial properties. Blueberry proanthocyanidins (B-PAC) are also known to have antiviral properties against human norovirus surrogates. A previous study showed that 5 mg/ml B-PAC can reduce AiV titers in suspension from ~5 log PFU/ml to non-detectable levels after 24 h at 37ºC.

Objective

This study aimed to determine the effect of B-PAC in apple juice (AJ) and milk as model systems and also under simulated gastric conditions against AiV over 24 h at 37ºC.

Methods

Treatments were prepared by mixing AiV at ~5 log PFU/ml with B-PAC at 2, 5, or 10 mg/ml prepared in either 10% ethanol, apple juice (AJ), 2% milk, simulated gastric fluid (SGF, pH 1.5), simulated intestinal fluid (SIF, pH 7.5), or AiV was mixed with individual controls (phosphate buffered saline (pH 7.2), malic acid (pH 3.0), apple juice (pH 3.6), and 2% milk)) and incubated over 24 h at 37ºC, followed by standard plaque assays. Data obtained from three replications run in duplicate were statistically analyzed.

Results

AiV was reduced to non-detectable levels after 30 min at 37ºC with both 1 and 2 mg/ml B-PAC in apple juice, while B-PAC at 2 and 5 mg/ml B-PAC in milk after 24 h at 37ºC caused AiV reductions of 0.52 and 0.84 log PFU/ml, respectively. AiV titers were reduced to non-detectable levels with B-PAC at 5 mg/ml in SIF after 30 min, while AiV did not survive in SGF at pH 1.5 by itself.

Conclusion

Overall, B-PAC shows potential to reduce AiV titers in food matrices (albeit at lower levels in 2% milk) and under simulated gastric conditions to decrease the risk of AiV-related illness and alleviate disease symptoms.

Hepatitis A virus inactivation by curcumin and heat

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Introduction

Foodborne outbreaks of hepatitis A virus (HAV) are linked to inadequate processing and improper food-handling conditions. Spices that are natural "generally recognized as safe" substances are used for flavour and antimicrobial properties worldwide. HAV is reportedly more heat resistant than most non-sporeforming bacteria and enteric viruses. Heat-sensitization of HAV using natural spices could reduce the time-temperature inactivation parameters during food processing to ensure food safety. Curcumin is a spice derived from *Curcuma longa* L. rhizomes known for health benefits as an anti-inflammatory, immunomodulating and antimicrobial agent.

Objective

The objective of this study was to determine and compare the D-values at 56, 60, 65 and 68ºC and z-values of HAV with and without curcumin.

Materials and methods

HAV at approximately 7 log PFU/ml was diluted in phosphate buffered saline (PBS) and directly added to one set of 2-mL sterile glass vials (as controls), with another set containing added 100 µL curcumin (400 µM). All vials were placed in a circulating waterbath heated at 56, 60, 65 and 68ºC. After reaching the come-up time (54 s, 60 s, 75 s, 86s for 56, 60, 65 and 68ºC, respectively), vials were treated for 0 to 10 min and immediately cooled on ice. Survivors were enumerated after ten-fold serial dilutions in cell-culture medium containing 8% bovine calf serum (BCS) using plaque assays on confluent host cells in 6-well plates. Each treatment in duplicate was replicated thrice and data were statistically analyzed.

Results

D-values of HAV in PBS were 9.21±0.24, 2.63±0.13, 1.8±0.08, and 0.67±0.19 min for 56, 60, 65, and 68ºC respectively, with a z-value of 11.65ºC using the linear model. D-values of HAV with curcumin were lower ranging from 8.22±0.18, 1.78±0.17, 1.43±0.12, 0.55±0.07 min for 56 to 68ºC respectively, with a z-value of 11.53ºC. These D-values with and without curcumin were significantly different for all temperatures ($p<0.05$) except 68ºC.

Conclusion

Reduction in D-values of HAV were observed overall using curcumin, indicating that lower heating temperature and time regimes to inactivate HAV can be potentially achieved by the addition of GRAS substances.

Persistence of norovirus in berry smoothies

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Introduction

Norovirus (NoV) is the most common causative agent of viral foodborne outbreaks. In 2016, 22 foodborne NoV outbreaks were reported in Finland. Berries are regarded as a high-risk food for NoV. On the other hand, fresh berry smoothies have become popular in recent years.

Objective

Our objective was to study if NoV can survive in berry-milk smoothies. The effect of time and temperature to the persistence of MuNoV in smoothies was studied.

Materials and Methods

Smoothie was prepared mixing milk, banana and raspberries or blueberries in the blender and then it was spiked with MuNoV. Infectious virus load in smoothie was tested after it was stored for 1 h at room temperature, 24 h at 37°C, or at 24 h at 4°C. In addition, the stability of virus in buffer, when incubated in refrigerator, was followed for three weeks. RAW macrophage cell cultivation and $TCID_{50}$ method was used for determination of the virus amount in 96-cell plates. Cytopathic effect in wells was followed by microscopic examination. Real-time reverse transcription (RT)-PCR from the cell supernatant was employed in unclear cases.

Results

MuNoV was found to remain stable in berry smoothies. When virus-spiked smoothie was incubated in refrigerator, $TCID_{50}$-values remained relatively constant, but a decrease of more than 80 % of the infectious MuNoV was observed at 37°C. When virus was stored in buffer at 4°C, the amount of both infectious MuNoV and MuNoV RNA reduced to a half of the original.

Conclusions

Our results suggest that berry smoothies may cause foodborne infections if NoV-contaminated berries are used for their preparation.

Two-year monitoring of F-specific RNA bacteriophages in Orne River (France)

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Objectives

The occurrence and propagation of faecal-derived viruses in streams constitute a major public health issue. However, little *in situ* information is currently available on the transport and dynamic of viruses in water, including the role of each stream compartment (water column, suspended solid particle and riverbed sediment). Viral particles can be considered as colloids in water and its fate is mainly driven by environmental factors. The objective of this study is to determine if there is a link between the concentration of F-specific RNA phages (considered indicators of faecal and viral pollution) and hydrological and meteorological parameters (e.g. discharge, antecedent precipitation and radiation) to better understand the dynamic, transport and seasonal distribution of viruses in the stream. We hypothesize that if we are able to decipher such relationships, we might be able to predict their concentration at a sampling point downstream of the faecal source.

Methods

Water samples were weekly collected at two sampling sites along the Orne River (France) during a two-year period. F-specific RNA bacteriophages (FRNAPH) were detected and quantified using an infectivity assay. A genogroup identification was also performed using RT-qPCR assay. In parallel, physical-chemical (water temperature, conductivity, turbidity), hydrological (flow rate, suspended solid particles) and meteorological (solar radiations, antecedent rainfall, air temperature) variables were measured to investigate the impact of environmental conditions on phage transport. The occurrence of FRNAPH on suspended solid particles was also investigated to estimate the extent to which they are attached to settleable particles.

Results and Conclusions

The results showed a different level of faecal pollution for the two sampling sites, with a higher concentration at the upstream site. The genogroup diversity was also highly superior at the upstream site. Among all the parameters studied, water temperature was identified to be the main factor responsible for the decrease of FRNAPH concentration along the stream. Interaction with suspended particles was negligible and therefore had no significant effect on virus transport. Treatment of all gathered data is currently in progress for the model.

Fate of bacteriophage during anammox side stream treatment of wastewater effluent

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Objectives

Following wastewater treatment at Tres Rios WWTP in Tucson, Arizona, biosolids are dewatered via centrifugation that results in ≃ 0.5 mgd of effluent (i.e. centrate) with high concentrations of ammonium (NH_4) at ~1000 ppm which must be re-treated before discharge into the environment. This project evaluates an alternative method of removing the NH_4 from the centrate effluent – namely anaerobic oxidation of ammonia, or anammox. We are evaluating the influence of anammox on bacteriophages and ultimately human pathogenic virus inactivation. Specifically, we will evaluate the incidence of FRNA-G1 bacteriophage that leach from the biosolids into the aqueous phase during centrifugation. Subsequent analysis of the centrate following anammox treatment will allow for an evaluation of virus inactivation levels due to the anammox process and high ammonium concentrations.

Methods

Mid-scale (173L) moving bed biofilm reactors (MBBR) have been established at the WEST Center for the evaluation of viral fate through anammox systems. Quantitative polymerase chain reaction (qPCR) and cultural assays will be utilized to assess total levels and infectivity, respectively of FRNA-G1 bacteriophage in the centrate effluent (both pre- and post-anammox).

Conclusions

These data will quantify the viral load associated with the biosolids dewatering process, and whether these viruses are inactivated or removed during the anammox process. The study data be used as a preliminary assessment as to viral fate through the anammox system. Future work may include the assessment of human pathogenic viruses through various anammox systems following the completion of this project. Additionally, the potential for pepper mild mottle virus and FRNA-G1 bacteriophage (both non-human viruses) to be utilized as conservative indicators for the fate of human enteric viruses during the annamox process.

Assessing the reduction of naturally occurring viruses by soil aquifer treatment of treated wastewater effluent

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Objective

Soil aquifer treatment (SAT) is a type of managed aquifer recharge in which water is intentionally infiltrated through the soil into groundwater. In many parts of Arizona, SAT of treated wastewater effluent is implemented for non-potable purposes. SAT exhibits removal of chemical and microbial contaminants, such as viruses, from effluents due to physiochemical properties of the soil. Virus transport models have been relied upon to estimate enteric virus removal due to past difficulties in virus recovery and detection. Because of the heterogeneity of subsurface strata, the accuracy of these models for viruses is unknown. The objectives of this project are to: (1) Determine virus removal efficiency of common human viruses in wastewater by SAT, (2) Assess the abilities of non-human viruses as a potential indicator organisms of virus removal, and (3) Compare the performance of newly operational SAT sites to those than have been in operation for longer durations.

Methods

Groundwater from three operational SAT sites at Tucson Water Sweetwater Reclamation Facility were sampled and analyzed for the presence of viruses throughout the duration of one year. Large volume samples were concentrated using a series of virus-adsorption-elution (VIRADEL) and centrifugal ultrafiltration steps. Detection was accomplished using quantitative polymerase chain reaction (qPCR). Viruses of interest included: Adenovirus, Enterovirus, Pepper Mild Mottle Virus, and CrAssphage. Log reductions were calculated using the concentration of viruses present in infiltrated treated wastewater effluent.

Results/Conclusion

A 3-5 log reduction of all viruses of interest during SAT was revealed. Non-human viruses have been detected in groundwater from the newly operation SAT site, whereas samples from older sites fall below detection limits. Results have indicated that duration of operation does not seem to influence virus removal efficiency, however other factors, such as infiltration rates, might have a greater influence on virus transport through soil to groundwater.

Reduction of human and plant viruses at a constructed wetland in Arizona, USA

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Objectives

This study aimed to determine the reduction of human and plant viruses at a constructed wetland (CW) receiving effluent of a wastewater treatment plant (WWTP) in Arizona, USA.

Methods

Water samples were collected at inlet, middle, and outlet points of the CW (n = 10 each), along with influent of the WWTP (n = 6). Viruses were concentrated using the electronegative membrane-vortex method followed by ultrafiltration. Viral DNA and RNA were extracted from water concentrates, the RNA was reverse transcribed, and five human viruses (noroviruses of genogroups I (NoVs-GI), enteroviruses (EVs), Aichi virus 1 (AiV-1), human adenoviruses (HuAdVs), and JC polyomaviruses (JCPyVs)) plus two plant viruses (pepper mild mottle virus (PMMoV) and tobacco mosaic virus (TMV)) were quantified using quantitative PCR. *Escherichia coli* was also tested as an indicator bacterium using Colilert (IDEXX Laboratories).

Results

PMMoV was detected with the highest positive ratio (100%, 36/36), followed by TMV (61%, 22/36), AiV-1 (39%, 14/36), EVs (31%, 11/36), HuAdVs (25%, 9/36), and JCPyVs (17%, 6/36). None of the samples were positive for NoVs-GI. Mean reduction ratios between the inlet and middle points were >3.77 log (n = 4) and >3.15 log (n = 4) for HuAdVs and JCPyVs, respectively, demonstrating that these viruses can be efficiently reduced by the CW. AiV-1 showed a low mean reduction of 1.21 log (n = 5) between the inlet and outlet points; however, AiV-1 was considered insufficient as an indicator virus because it was not detected in the outlet samples. On the other hand, the concentrations of PMMoV in the CW were relatively high and fairly constant. Interestingly, increased concentrations of *E. coli* were observed throughout the CW system.

Conclusions

PMMoV was considered a suitable indicator of virus reductions by the CW, based on its high and stable concentrations.

Strain-specific Differences in Response of Human Noroviruses to pH Challenge

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Introduction

Disinfection is an important tool for controlling norovirus outbreaks. Marketed disinfectants against human norovirus are formulated across a wide pH range, from highly acidic to highly alkaline. Because most studies on the impact of disinfectants against human norovirus use cultivable surrogates, and some surrogates are more sensitive to extremes of pH, a comprehensive analysis of the effect of pH on common human norovirus strains can better inform surface disinfectant formulation for outbreak control.

Methods

Clarified 20% suspensions of human feces confirmed positive for GII.4 Sydney or GI.6 were used either directly or after chloroform extraction for additional clarification. Aliquots were exposed to buffered solutions (ranging from pH 3 to 13) for contact times of 30 minutes or 2 hours. Following neutralization the suspensions were subjected to RNA extraction (with or without RNAse pre-treatment) and subsequent RT-qPCR. Log_{10} reduction was calculated based on genomic copies; all experiments were repeated in triplicate.

Results

Neither GII.4 Sydney nor GI.6 demonstrated significant log_{10} genome copy number reduction at acidic or neutral pH at an extended 2 hour contact time in suspension. Solutions with alkaline pHs had variable efficacy; whereas GI.6 showed between 1.5 and 3.5-log_{10} reduction for solutions at pH 10 and 12-13 at a 30 minute contact time, GII.4 Sydney showed lesser degrees of reduction (0.4-1.5-log_{10}) at pH 12-13 and none at pH 10.

Conclusions

Disinfectant formulations made at pH 12 or above may provide synergistic anti-noroviral activity from an added pH effect, which could reduce necessary disinfectant concentrations or contact times. Tailoring pH values to specific virus strains may enhance this effect.

In vitro and in vivo efficacies of hand sanitizers against human norovirus

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Introduction

Human norovirus is the leading cause of acute non-bacterial gastroenteritis and foodborne disease. Contaminated hands play a significant role in the spread of the virus, particularly during food handling. Hand sanitizers are purported to interrupt norovirus transmission but their antiviral efficacy is poorly characterized.

Methods

The current outbreak strain (GII.4 Sydney), obtained as a deidentifed stool specimen was suspended 20% in PBS and used as inoculum. Virucidal suspension assays for *in vitro* studies (ASTM International standard E1052) and *in vivo* fingerpad studies using human volunteers (n=10) (ASTM E1838) were done with exposure times of 30 and 60 sec. Virus concentrations before and after exposure to the sanitizers, and including controls, were evaluated by RT-qPCR preceded by RNase treatment.

Results

The benchmark treatment (60% ethanol) produced 0.9±0.09 $\log_{10}$ and 1.7±0.46 $\log_{10}$ reduction in norovirus genome equivalent copies (GEC) after 60 sec by *in vitro* and *in vivo* methods, respectively. Comparatively, a commonly used hand sanitizer containing 70% ethanol produced 2.5±0.2 $\log_{10}$ GEC reduction after 60 sec by suspension test, and 2.0±0.4 $\log_{10}$ reduction using the fingerpad method. Between benchmark. With a benzalkonium chloride (BAC)-based product, 0.8 ±0.2 and 1.3±0.1. $\log_{10}$ GEC reductions were observed after 60 sec for *in vitro* and *in vivo* assays, respectively. A new hand sanitizer containing 80% ethanol produced 4.1±0.3 $\log_{10}$ reduction after 60 sec by *in vivo* study and was significantly greater than benchmark and all the other products tested.

Conclusions

These results demonstrate that (BAC)-based product is not effective against human norovirus, nor alcohol by itself but effective formulations alcohol-based can achieve significant reduction of norovirus on fingers.

Hidrodynamic cavitation, a powerful tool for waterborne virus inactivation

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Objectives

Water for human consumption, or for human related activities (i.e., irrigation), should preferably be free from microbiological hazards. Modern molecular technologies are increasingly relating human and plant viruses to waterborne disease outbreaks or crop losses. The USA EPA states that a proper water disinfection method should reduce the viral load in 4 logs, while new European legislation is in preparation. The purpose of our research is to implement novel, clean and efficient methods, hydrodynamic cavitation for inactivation of waterborne viruses.

Methods

To establish a proof of principle we used two different cavitation reactors, of 3 ml and 1 L volumes, respectively. Tap water was spiked with bacteriophage MS2 at high (10exp8,8 pfu/ml) and low (10exp2,7 pfu/ml) concentrations, followed by exposure to a number of cavitation cycles. The effect of hydrodynamic cavitation on the viral viability was assessed with the double layer agar assay. Proper controls were used to ensure the effect on viral infectivity was due only to cavitation.

Results

1000 cavitation cycles (cca 1 hour of exposure), reduced in more than 4 logs the infectivity of MS2 spiked at high concentrations in both the low scale and medium scale reactors. Low MS2 concentrations were completely inactivated after 416 and 208 cavitation cycles in the medium and low scale reactors, respectively.

Conclusions

These results confirm hydrodynamic cavitation as a valuable, clean and efficient tool for waterborne virus disinfection. In the frame of a recently awarded ERC consolidator grant CABUM (Dr. Matevz Dular), we are going to explore deeper the cavitation mechanisms of viral inactivation and expand the technology to other human and plant viruses, as well as bacteria. This will open doors for designing higher scale cavitation devices to be tested at industrial level such as wastewater treatment plants.

Non-oxidizing agent to control viruses and other pathogens of public health concern

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The dynamics of infection spreading throughout a population necessitates new and safe disinfectants as resistance develop and effectiveness attenuate. The biocidal properties of guanidine compounds have long been recognized and Polyhexamethylene biguanide hydrochloride (PHMB) is well established widely accepted as biodegradable, non-corrosive and nontoxic disinfectant when used as directed. Another guanidine compound, Polyhexamethylene-Guanidine Hydrochloride (PHMGH) has been demonstrated to have biocidal properties comparable or more effective than PHMB. PHMGH studies have shown that in water solution it has fungicidal as well as bactericidal activity against both gram-positive and gram-negative bacteria including effectively destroying Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, spore forming bacteria such as *Clostridium difficile* at biocide concentrations well below what is required for other disinfectants. However, not much information is available on the efficacy of PHMGH against viruses and algae.

The focus of this work was to demonstrate the biocidal efficacy of PHMGH in a variety of applications covering diverse scenarios including controlling viral pathogens on surfaces and algal blooms in surface water. Bacteriophage MS2 and P22 were used to evaluate the viricidal efficacy of PHMGH. Algaecidal efficacy was tested using Chlorella and Cyanobacteria (*Synechocystis* PCC 6803). Viricidal efficacy of 0.05% PHMGH was tested at 15-minute and 30-minute contact time.

After 15-minute contact with 0.05% PHMGH, log reduction for MS2 and P22 were 0.25 and 0.15 respectively. Under similar conditions, after 30-minute contact time inactivation of MS2 and P22 were 0.35 and 0.25 logs, respectively. After 12- hour exposure to 0.05% PHMGH, only 4.4% of green algae and 52% of cyanobacteria were viable. Whereas after 48- hour exposure 99.4% green algae and 100% Cyanobacteria were killed. Based on the residual and longitudinal studies, the product has a clear biocidal effect. The product has shown potential as surface disinfectant, which need further investigation of its inefficacy under different application scenarios.

Developing buffer zones for norovirus in shellfish production areas using chemical tracers, environmental microbiology and satellite remote sensing

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Purpose/Objectives

Bivalve shellfish grown in waters contaminated with sewage pollution can accumulate norovirus (NoV) and, if consumed raw, can cause gastroenteritis in consumers. Commercial production of these shellfish needs to ensure that this risk is satisfactorily addressed, through appropriate choice of production area and adequate processing. Internationally, food hygiene controls aimed at preventing shellfish-related illness prescribe standards based on faecal indicator bacteria. To reduce the risk of illness, many countries have implemented additional control measures for shellfish production areas (SPAs). In the USA, the Shellfish Sanitation Program recommends that all SPAs impacted by sewage discharges adjacent to the production area be prohibited to shellfish harvest (buffer zones). In the EU, there is no parallel legislation although some countries have implemented buffer zones around sewage outfalls, harbours and rivers.

Materials/Methods

Under Project SEAFOOD^{TOMORROW}, a protocol that combines dye tracing, environmental microbiology and satellite imagery is being developed to model the relationships between NoV in shellfish and the dilution of sewage effluents in SPAs. The purpose of the models is to inform the policy community and shellfish farmers on appropriate criteria for delineating buffer zones necessary to mitigate viral risks.

Results/Conclusions

Initial results indicate significant associations between total concentrations of NoV and sewage effluent dilution in the SPAs. Reference microbiological concentrations have been obtained for dilution ratios of estuarine water:sewage effluents. A good agreement has been found between turbidity fields of river plumes derived from Landsat 8 imagery and the *in situ* microbiological data. The model outputs will be used to validate a dynamic approach for buffer zones that is applicable to coastal sites with different pollution source impacts and hydrographic characteristics.

Hepatitis A outbreaks and environmental circulation of genotype IA strains in metropolitan São Paulo, 2017-2018

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Hepatitis A virus (HAV) is the major cause of enterically transmitted infectious hepatitis. The fecal-oral route is the primary transmission path of HAV infection, including person-to-person contact and food and water contaminated. Since 2016, an unusual increase in number of hepatitis A cases has been reported among men who have sex with men (MSM) by low endemicity countries. At São Paulo city the number of confirmed Hepatitis A cases (Ig-M immunoassay) increased from 64 in 2016 to 698 in 2017, affecting mainly adults aged between 18 and 39 years (80%) and males (87%). A total of 41 % cases were reported as sexually transmitted and 11% were related with water and food contaminated. To support epidemiological surveillance, the present study aimed to monitor the presence of HAV in 20 urban sewage samples of São Paulo city, collected between November 2017 and May 2018 in the central region of the city, where the most of cases were confirmed. One liter samples were concentrated by PEG and HAV were quantified by qPCR. VP1/2A junction region of positive samples were sequenced. HAV were detected in 95% (19/20) of the samples, with mean viral loads of 1.61×10^5 ($\pm$ SD 1.15×10^6) genome copies (GC/L). Out of the 19 positive samples, 8 submitted to sequencing were confirmed as HAV subgenotype IA, all of them close related (99-100% nucleotide identity). The Blast search revealed that these sequences have high homology ($\geq$ 99%) with the sequences obtained in European countries (Italy, France, Spain), most of them described in recent outbreaks associated with MSM. Our results point to the risk of high dissemination of HAV genotype IA in the environment and the need to establish urgently measures for outbreak control among MSM. The State Health Department has promoted vaccination to individuals at risk group.

Foodborne viruses - molecular epidemiology in the Czech Republic

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The number of foodborne outbreaks caused by viruses increased in the EU. In spite of it, the viruses are rarely determined as causes of food- or waterborne outbreaks in the Czech Republic (CZ). The unpreparedness of the national surveillance system for foodborne diseases was revealed during a norovirus (NoV) waterborne outbreak (4,021 human cases) in 2015, Prague. The main problem was the amble determination of the causative agents and the source of the contamination.

Therefore national project focused on the most important foodborne viruses, e.g. NoV, hepatitis A virus (HAV) and hepatitis E virus (HEV) was initiated in 2017. The main aim of the project is molecular typing of foodborne viruses from clinical material and food- or waterborne outbreaks. Sequence and phylogenetic analyses is used for epidemiological studies as well as for the creation of a database of viral strains circulating in the CZ.

Up to date a total of 400 partial sequences of HAV (VP1/2A) and 57 of NoV (ORF1/ORF2, RdPd) originating from human patients and environment, and 284 of HEV (ORF1) from human patients, domestic pigs and wild boars were analysed. Sequence analyses confirmed by epidemiological data provided clear evidence of the relationship between the agents and resulting infection; zoonotic transmission of HEV, NoV food- and waterborne outbreaks, discrimination between related HAV outbreaks and transmission of HAV by blood transfusion.

The results emanating from this project improves our understanding regarding the prevalence and ecology of foodborne viruses in the CZ and strengthen the cooperation between human and veterinary laboratories and Regional Public Health Authorities.

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Evaluation of crAssphage-based qPCR marker with human fecal indicators in Lake Pontchartrain, Louisiana

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Introduction

Water quality in Lake Pontchartrain was deteriorating and recreational activities along the beach were restricted by the end of the 20th Century. In the past, we used human specific marker to identify the source(s) of human fecal pollution. However, these markers lack sensitivity and do not correlate with viral indicators. There is a new novel crAssphage marker and it is believed to be highly human specific and abundant in sewage as indicated by recent publications.

Objectives

Our goal is to evaluate this crAssphage marker with other source tracking markers to identify the source of fecal contamination in Lake Pontchartrain, Louisiana.

Methods and Results

Water samples were collected over an eight-month period at ten locations along the lake in 2016 and 2017. *E. coli* and *Enterococcus* were detected in 90.6% (culture) and 97.5% (qPCR), 95.8% (culture) and 91.8% (qPCR) of water samples from all sampling sites, respectively.
Significant positive relationship between *E. coli* and *Enterococcus* results was observed for both qPCR and culture methods. HF183 marker was detected in 94.3% water samples (149 of 158), with concentrations ranging from 29.0 to 6073.5 GC/100 ml and from 129.8 to 38,465.6 GC/100 ml in summer and winter, respectively. The crAssphage markers are also present in 40 samples that we have processed so far. We are still in the process of analyzing all our samples and this poster presentation will reveal the concentrations and correlation with fecal indicators especially HF183.

Conclusions

Our results indicate that significant rainfall events have the potential to supply considerable loads of faecal bacteria to lake waters. This study is also the first to utilize this marker for monitoring faecal contamination in Lake Pontchartrain, Louisiana.

Algal Phages – a Threat to the Health of Algal Production Operation for Food and Biofuel Application

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Microalgae and cyanobacteria has been used for nutritional purposes for centuries. In the last few decades, developments in biotechnology have resulted in the introduction of algae based value-added products for use as food and in cosmetics. The bioactive components such as proteins, essential fatty acids, polysaccharides, vitamins, and minerals are harvested form large commercial algal production operations. These large-scale operations face many production challenges including naturally occurring algal phages. We report the detection and quantification of algal phages in natural algal populations in freshwater habitats and in the laboratory cultures of microalgal strains. Culture-based and electron microscopy techniques were used to characterize the algal phages. In addition, bench-scale experiments were performed to study the impact of viral infectivity on micro-algal production in laboratory-scale bioreactors.

All field samples tested positive for algal phages with various levels of infectivity against *Synechocystis* PCC 6803 and the environmental isolates of microalgae. The viral attachment to algal cells were observed under transmission electron microscopy (TEM). The shape and size of the viral particles were determined by obtaining electron micrographs. The viruses detected were of similar size (~ 50-60 nm) and shape (icosahedral virion). After viral infection, reduction in growth of microalgae was quantified by using optical density measurements and the transition of the culture from dark green to yellowish green. The infection resulted in 48% reduction in the biomass of the infected algal culture in 22 days. This study lead to the conclusion that the algal phages are prevalent in natural environment and may cause infections in broad range of microalgal species. In addition, phage infection can be a serious threat to the yield and the quality of bioactive compounds in commercial microalgae production operation.

Whole genome sequencing reveals prophage sequences present in Escherichia coli isolated from pristine environments

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Purpose/Objectives

Temperate bacteriophages are involved in lateral transfer of genes coding for novel enzymes that may give the recipients metabolic diversity or may result in an alteration of their physiology. Analyzing these phages could provide information on bacterial fitness and evolution. The present study focuses on studying and characterizing prophage regions present in environmental E. coli isolates. We will focus on the possible impact these sequences have on genome arrangement in the environment.

Materials/Methods

Whole genome sequencing was carried out on E.coli isolated from pristine sites. Genomic data were analyzed using online platforms such as: PHASTER for phage identification and CRF: CRISPR Finder by Random Forest was used to determine CRISPR sequences and arrangement of immunity sequences.

Results and Conclusions

We successfully determined the presence of more than one prophage in the E. coli pangenome, suggesting that phages are important entities of diversity. Phage-like elements have been identified in all isolate genomes resulting in an unique phage architecture. A total of 46 phages were identified within the bacterial genomes. These phages were either classified as intact 17, questionable 5 or incomplete 24. Our results suggest the presence of CRISPR spacers as well as that of Cas proteins. Most isolates showed a distinct phage region, as well as unique CRISPR spacer arrangements, in some case almost complete heterogenicity to the other phages found. Data demonstrate the presence of a diversity of CRISPR cassettes. However, more studies are needed in order to characterize them and determine how these may play a role in phage immunity. In conclusion, this study confirmed the role of bacteriophages in genome rearranging maps. Additionally, this is the first report on the presence of these sequences in E. coli isolated from pristine environments.

Chemical Induction Followed by High-Throughput Sequencing: Prophage Presence, Host Range and Promiscuity as Possible Roles in Genome Evolution

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Objectives

Viruses are the most abundant biological entities, however little is known about their role in the environment, specifically in bacterial evolution. We hypothesize that *Enterococci* spp. prophage regions may play a significant role in genome architecture resulting in the acquisition of traits and genome diversity. We tried to determine the broad host-range of induced lysogenic phages and to study prophages regions in *Enterococci* spp. isolated from pristine sites in Puerto Rico.

Methods

Lysogenic enterophages in *Enterococcus* spp. pristine site isolates were induced and the supernatant tested against 8 type strains of *Enterococcus*. Eighteen induced isolates were selected for High-throughput sequencing, based on their promiscuity. Phage identification pipelines PHASTER and CRISPRCAS were used to identify prophage regions and CRISPR sequences in the draft genomes.

Results and Conclusions

Prophage induction indicated the presence of specific range as follows: 52.90% for *E. faecalis*, 52.90% *E. faecium*, 17.70% *E. hirae*, 13.72% *E. durans*, 9.80% *E. dispar*, 3.92% *E. pseudoavium* and 3.92% *E. gallinarum*. The infection of induced temperate phages revealed the ability to infect various hosts within the same genus. At the genomic level, abundant prophage region sequences were observed; unexpectedly our results showed the presence of phages usually reported as infecting other genera. CRISPR sequences were detected in all our genomes, however more studies are needed to determine immunity sequences. The presence of lysogens, supports the hypothesis that phages may be key in the resulting genomic heterogenicity in genome architecture (at least amongst members of the same genus). Our data clearly demonstrate that lysogenic enterophages have a wide host range and may be promiscuous leading to a favorable gene exchange in the environment.